ANAEROBIC BIODEGRADATION OF LINEAR MODEL NAPHTHENIC ACID UNDER DENITRIFYING CONDITIONS

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by

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ABSTRACT

The oils sands have become significant source of energy supply and a major economic driver for Canada. Due to the increase in production of oil, the activity of surface mining to extract oil sand increases. To carry out continuous operation of extracting bitumen from oil sands, industry uses large volumes of freshwater. Generation of produced oil sand process water which has to be treated prior to discharge in environment has become a significant issue of environmental concern. The presence of naphthenic acids was found to be the source of toxicity in the process water associated with oil sands. This together with necessity of sustainable use of water, the need develops for the effective treatment technologies such as bioremediation of process water contaminated with these naphthenic acids.

In the present work, anaerobic biodegradation of a linear model naphthenic acid (Octanoic acid) under denitrifying conditions was studied. The microbial culture utilized in the biodegradation of model naphthenic acid compound was already developed in the laboratory and acclimatized to the anaerobic conditions. Octanoic acid was used as the organic substrate and potassium nitrate as electron acceptor in the experiments. The batch and continuous stirred tank bioreactor experiments were performed to evaluate the biokinetics of biodegradation of octanoic acid under denitrifying conditions. In the batch experiments the influence of concentration of octanoic acid and nitrate, and temperature were studied. In the continuous experiments effect of loading rate on removal were observed. Finally, the results revealed the efficient removal of octanoic acid following with the process of denitrification.

Keywords: Naphthenic acids, Anaerobic biodegradation, Denitrification, Bioremediation

Table of Contents

List of Figures

List of Tables

Abbreviations

4MCHAA - 4-methylcyclohexane acetic acid

cis-4MCHAA - *cis*-isomer of 4-methylcyclohexane acetic acid

CSTR - continuous stirred tank reactor

ESI - electrospray ionization

FID - flame ionization detection

FTIR - fourier transform infrared

GC - gas chromatography

IC – ion exchange chromatography

HPLC - high-performance liquid chromatography

LSI - liquid secondary ion

MS - mass spectrometry

MEUF – micellar-enhanced ultrafiltration

NA - naphthenic acid

NAs - naphthenic acids

OD - optical density

OSPW – oil sand Process water

QTOF - quantitative quadrupole time of flight

RO - reverse osmosis

TAN – total Acid Number

trans-4MCHCA - 4 methyl-1-cyclohexane carboxylic acid

trans-4MCHAA - *trans*-isomer of 4-methylcyclohexane acetic acid

μ - specific growth rate

Chapter 1

INTRODUCTION

Crude oil is a complex mixture of hydrocarbons, basically composed of aliphatic, aromatic and asphaltene fractions along with nitrogen, sulphur and oxygen-containing compounds. The constituent hydrocarbon compounds are present in varied proportion resulting in great variability in crude oils from different sources (Speight, 1999). There are several reports indicating the recalcitrance and potential health hazards of the different constituents of crude oil (Kanaly & Harayama, 2000). Naphthenic acids (NAs) comprised of a large collection of alkyl-substituted alicyclic and cyclic carboxylic acids found in hydrocarbon deposits (oil sands bitumen, and crude oils). They are described by the general formula $C_nH_{2n+Z}O_2$, where *n* indicates the carbon number and *Z* is zero or a negative, even integer that specifies the hydrogen deficiency resulting from ring formation (Brient et al., 1995; Clemente et al., 2005).

NAs are naturally present in oil sands in Canada (north Alberta) which represent second largest proven oil deposits in the world, after reserves in Saudi Arabia. There are three major oil sand deposits in the north Alberta namely Athabasca-Wabiskaw, the Cold Lake, and the Peace River oil sands deposits (Allen, 2008). It is estimated that about 27.6 billion m^3 (175 billion barrels) of bitumen exist in the northern oil sands (Huang & Mehdi, 2011). To meet the increased global demand of oil, recovery and processing of this unconventional fossil fuel, recovery of bitumen from oil sands has reached 1 million barrels per day, and is projected to be triple over next decade (National Energy Board 2006).

With the rapid expansion of oil sands industry and oil production, the continuous supply of fresh water is needed for meeting the surface mining operations. Under the current surface mining operations, bitumen is extracted from the ore using variations of the Clarke caustic hot water extraction process (Han et al., 2009). Presently, each barrel of oil produced from surface mining consumes 3 barrel of fresh water (Syncrude Canada, 2004; Suncor Energy, 2005; Shell Canada, 2005). Generation of these large volumes of oil sands process water (OSPW) consist mainly of solids (sand and clay), water, unrecovered bitumen, dissolved organic, and inorganic compounds. These aqueous slurries contain recalcitrant and toxic naphthenic acids which are most significant environmental contaminants. Under the "zero-discharge policy", the oil sand companies do not release these process water containing naphthenic acids; retain them on large tailing ponds on the site to prevent their release to

aquatic organisms (Quagraine et al., 2005, Paslawski, 2008). In 2003, the estimated volume of tailings in the Athabasca region was approximated $4x10^8$ m^3 and the total volume is expected to increase to over 1 billion m^3 by 2020 (Paslawski, 2008). As the volume of tailing ponds is expanding, the continuous recycling of process water has deteriorated the water quality, and shortage of fresh water resources in oil sands operations, has led to considerable reclamation efforts of tailings accumulated with toxic NAs.

Over the past few years, many techniques and methods are being tried like microfiltration, ultrafiltration, micellar-enhanced ultrafiltration (MEUF), biodegradation and advance oxidation to remediate oil sands process water (OSPW). Among these bioremediation is considered to be most reliable and cost effective water treatment technologies.

Although many literatures report the biodegradation of naphthenic acids in the tailing ponds, surrogate naphthenic acids or commercial naphthenic acids, but most of the biodegradation studies are under aerobic conditions using various cultures. However, only few studies have focused on biodegradation under anaerobic conditions using surrogate naphthenic acids and production of methane was observed utilizing microorganisms from oil sands and sewage culture (Holowenko et al., 2001). As most of tailing ponds are anaerobic, further investigations of the factors controlling the anaerobic biodegradation kinetics are to be evaluated and enhancement of anaerobic *in-situ* or *ex-situ* process (novel bioreactors) is needed.

In current work, a mixed culture developed in the laboratory suitable for degrading NAs under aerobic conditions was used under anaerobic conditions using nitrate as an electron acceptor and octanoic acid as substrate. After the enrichment, the culture was utilized to biodegradation of the linear model NA compound (octanoic acid) under denitrifying conditions and kinetic studies have been investigated in batch and continuous mode of operation. Effects of NA concentrations and temperature on biodegradation have been assessed in the batch mode. To better determine the biokinetic coefficients and enhancement of biodegradation rate, a continuous stirred tank reactor (CSTR), with octanoic acid under denitrifying conditions was also operated.

Chapter 2
LITERATURE REVIEW

2.1 Naphthenic Acids

Naphthenic acids are a complex family of naturally occurring cyclic and acyclic carboxylic acids that are present in the acidic fraction of petroleum. NAs have the general chemical formula $C_nH_{2n+z}O_2$, where 'n' refers to the number of carbon atoms, and 'z' is zero or a negative number that refers to the hydrogen atom deficiency caused by ring formation (Holowenko et al., 2002; Clemente et al., 2005). They can be monocyclic, polycyclic or acyclic (Rogers et al., 2002). For example, NAs can be saturated, acyclic acids with 'z' equals to 0, monocyclic acids (z = -2), bicyclic acids (z = -4), or tricyclic acids (z = -6) etc. NAs mixture consists mainly of monocyclic acids as the carbon number in the range of 7 to 12 (n = 7 to 12), and multicyclic acids become predominate as carbon number increased above 12 (n >12) (Brient et al., 1995). Based on the current reports it seems that NAs with z = -4 predominate in Athabasca oil sands tailings ponds waste waters of Suncor and Syncrude (McMartin, 2003; Headley et al., 2004; Mishra, 2009). The acyclic components are highly branched, unlike fatty acids, although fatty acids fit the formula $C_nH_{2n+z}O_2$, for z = 0 (Rudzinski et al., 2002; Clemente et al., 2005). Figure 2.1 shows examples of naphthenic acids structures. Besides the carboxylic acid group, cyclic naphthenic acids are believed to be substituted with alkyl groups (R in Figure 2.1). Till date, no analytical technique is available for the assessment of this complex mixture of NAs. This diversity makes them challenging to study.

2.2 Commercial Uses of Naphthenic Acids (NAs)

More than two-thirds of the naphthenic acid produced is used to make metal salts, with the largest volume being used for copper naphthenates, consumed in the wood preservative industry (Brient et al., 1995). Other use of NAs and metal naphthenates includes in textile and paint driers, emulsifiers, surfactants, and adhesion promoters in tire manufacture (Clemente et al., 2004). Besides metal naphthenates, free naphthenic acids used in concrete additives, motor oil lubricants, asphalt-paving applications and ore flotation for recovery of rare-earth metals (Brient et al., 1995). Various industrial applications of naphthenic acids have been summarized in Table 2.1.

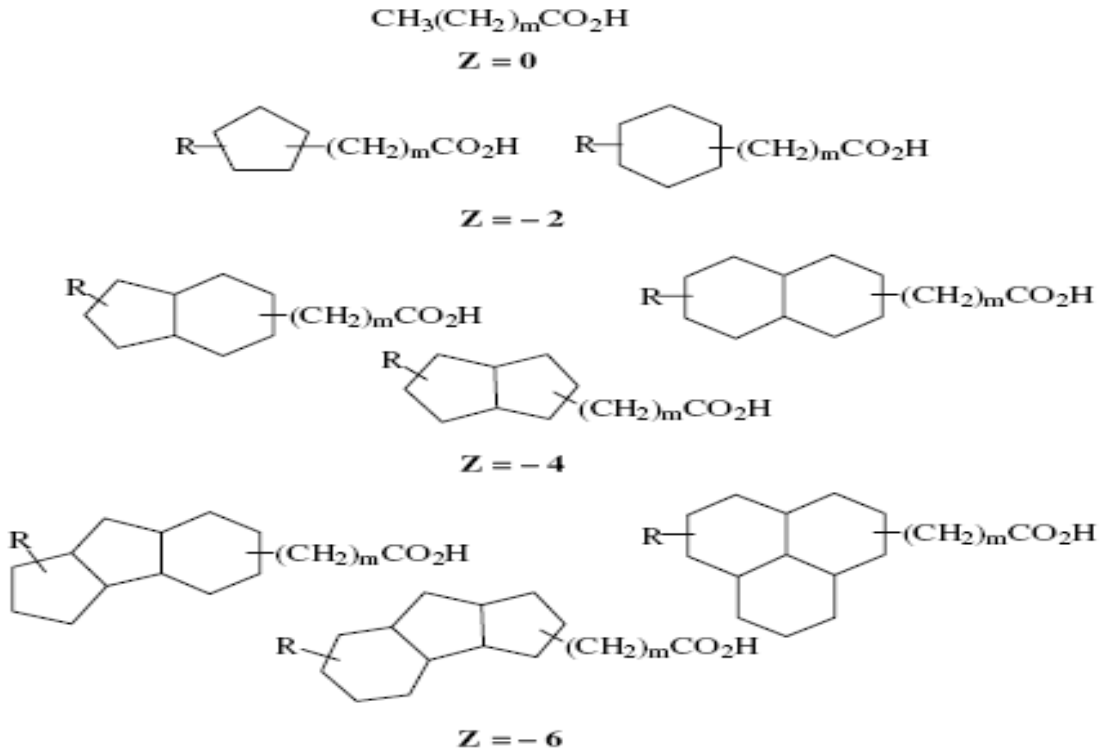


Figure 2.1 General chemical structures of naphthenic acids, where *Z* represents hydrogen deficiency, R is an alkyl chain, and m indicates the number of CH_2 units (Clemente et al., 2005)

Table 2.1 Industrial applications of naphthenic acids (Brient et al. 1995; McMartin, 2003).

Naphthenic acid metal salt	**Industrial applications**
Na salts	Emulsifying agent for agricultural insecticides Additive for cutting oils emulsion breaker in oil industry
Ca naphthenates	Additive for lubricating oil
Fe and Mn naphthenates	Fuel additives for improved combustion and reduced corrosion
Cu and Zn naphthenates	Wood preservatives
Co naphthenates	Curing agent in rubbers and resins Adhesion promoter of steel cord to rubber
Mn, Pb, Co, and Ca soaps	Oxidative catalysts

2.3 Physical and Chemical Properties of NAs

Naphthenic acids are a family of carboxylic acid surfactants primarily consisting of cyclic terpenoids used in source and geochemical characterization of petroleum reserves. (Headley & McMartin, 2004). Naphthenic acids are viscous liquids, with phenolic and sulphur impurities present that are largely responsible for their characteristic odour. Their colours range from pale yellow to dark amber (Brient et al., 1995). The polarity and non-volatility of naphthenic acids increases with molecular weight, giving individual compounds various physical, chemical, and toxicological properties (Headley & McMartin, 2004). The boiling points of naphthenic acids are in the range of 250 to 350 ^{0}C and this property makes them suitable for their application as alloys (Clemente et al., 2005).

NAs are chemically stable and have dissociation constants ranging between 10^{-5} and 10^{-6} (Brient et al., 1995), which is typical of most carboxylic acids (acetic acid = $10^{-4.7}$, propionic acid = $10^{-4.9}$, palmitic acid = $10^{-8.7}$) (Clemente et al., 2005). Chemically NAs behave like carboxylic acids and have acid strength similar to higher fatty acids (Headley & McMartin, 2004). Table 2.2 illustrates the various physical and chemical properties of naphthenic acids.

Table 2.2 Physical and chemical properties of naphthenic acids (Brient et al. 1995; Headley et al., 2002a; McMartin, 2003).

Parameters	General Characteristics
Colour	Pale yellow, dark amber, yellowish brown, black
Odour	Primarily imparted by the presence of phenol and sulphur impurities; mostly hydrocarbon odour
State Viscous liquid	Viscous liquid
Molecular Weight	Generally between 140 and 450 amu
Density	0.97 and 0.99 g/cm^3
pKa	Between 5 and 6
Solubility	Between<50 mg/L at pH 7 in water Completely soluble in organic solvents
Boiling point	Between 250 and 350 ˚C

2.4 The Oil Sand Industry and Occurrence of Naphthenic Acids

Oil sands are deposits of bitumen, a heavy, black, thick, sticky form of crude oil. One of the largest oil sands (a mixture of sand, clay, and bitumen) deposits in the world is in the Athabasca Basin in north-eastern Alberta, Canada. Deposits are estimated to contain over 1.7 trillion barrels of bitumen and likely represent the largest accumulation of highly biodegraded crude oil source in the world (MacLean, 1998; Quagraine et al., 2005). Bitumen makes up about 10-12% of the actual oil sands found in Alberta. The remainder is 80-85% sand and clays and 4-6% water (Government of Alberta, 2004). The bitumen in the oil sand contains naphthenic acids, alkanes and aromatics, resins and asphaltenes.

Before bitumen can be upgraded into crude oil for refining stages, it must be separated from oil sands. Oil sands are mined from various depths and locations and contain different distributions of naphthenic acids, both in terms of composition and concentration. The presence of carboxylic acid content (mainly 2% and 90% tricyclic) in the Athabasca oil sands bitumen, make up the naphthenic acid fraction (Quagraine et al., 2005) and increases the oil acidity. For instance, processing of crude oil with such high content of NAs is a concern for oil sand processing companies because of their potential to cause corrosion. This corrosivity increases with the increasing total acid number (TAN) (Clemente et al., 2005). Thus, the petroleum products with such high acid number have generally less commercial value as compared to low acid number.

To circumvent these problems, bitumen is extracted from oil sands using Clarke caustic hot (79-93 ^{0}C) water extraction method to produce crude oil and the method also removes NAs in to water as naphthenates (Quagraine et al., 2005). These dissolved naphthenates are surfactants-organic compounds possessing both hydrophilic and hydrophobic components that reduces surface-tension and the other liquids, and plays a vital role in the extraction procedure (Schramm et al., 2000; Quagraine et al., 2005). To carry out the process of separation efficiently, large amount of water is needed and this extraction procedure produces large volume of slurry waste contaminated with naphthenic acids. It has been estimated that for each cubic meter of mined oil sands, a volume of 3m^3 of water and approximately 4m^3 of slurry waste consisting of sand, clays, organics, residual bitumen, and process water result as a by-product of the bitumen extraction process (Holowenko et al., 2002; Quagraine et al., 2005). NAs are significant contaminants in these aqueous slurries.

The other fractions of organic compound that are removed during the process include phenols, mercaptons, thiophenes, and cresols (Brient et al., 1995). Under the current environmental regulations, oil sand companies do not release any extraction waste from their leases, because of "zero-discharge policy", and thus oil sand process water are to be stored on-site large tailing ponds (Holowenko et al., 2002; Paslawski et al., 2008). These aqueous slurries containing NAs in the large tailing ponds can be acutely and chronically toxic to aquatic organisms. Although most of the water used for separation process is recycled and reused in the extraction process, there are a number of environmental concerns associated with water usage in oil sands industry. Currently, the NAs concentrations in the tailing ponds range from 40 to 120 mg/L (Schramm et al., 2000; Holowenko et al., 2002; Mishra, 2009).

Apart from increasing concentration of NAs in the tailing ponds, the major concern is the volume of fine tailings accumulating in the tailing ponds. Currently the calculated volume of tailing ponds is 300 million of m^3 and it is estimated that if process continues at this rate, the volume is expected to rise to over 1 billion of m^3 by 2025 (Lo et al., 2003; Quagraine et al., 2005).

With the increasing water demands due to the rapid development of oil sand projects, and reports calling for further regulation of water imports from the river, the reclamation of OSPW is an important challenge facing the oil sand industry today (Schindler et al., 2007; Han et al., 2009). Currently, no single remediation method is capable of reclaiming the OSPW, and thus method to target these volumes of fine tailing contaminated with NAs should be environmentally safe and economically feasible. Bioremediation of these wastewaters comes out to be the best option, which has the potential of treating the OSPW without hampering the biological systems in ecosystem.

2.5 Environmental Implications

NAs have chronic and acute toxicity to aquatic organisms. The reported toxicity of NAs is due to its surfactant characteristics (Rogers et al., 2002a; Quagraine et al, 2005). The toxicity content and complexity of NAs in bitumen varies with oils sands formation. To date, there is no analytical method that will separate the individual compounds found in the complex mixture of NAs. The toxicity is dominant in low molecular weight NAs rather than in high molecular weight NAs (Mackinnon, 2001), in addition to this Holowenko et al. (2002) suggests from their findings that a decrease in the proportion of lower molecular weight acids contributes to the reduced acute toxicity of the residual NAs.

Determination of the total concentration of naphthenic acids is not sufficient to explain the toxic effects, many research results demonstrate that the toxicity eventually can be related back to the concentration of individual NAs (Holowenko et al., 2002; McMartin, 2003; Paslawski et al., 2008). Both oil sands development and natural exposure to oil sands deposits may result in concentrations of hydrocarbons and other substances, in both the water and sediment, which cause stress to fish and other biota (Conly et al., 2001; Headley et al., 2001). Fresh tailings water from oil sands processing is acutely toxic to aquatic organisms (LC_{50}<10% v/v for rainbow trout) (Mackinnon et al., 1986) and mammals (oral LC_{50}¼3.0 g/kg body weight) (USEPA, 1994). The most recent mammalian toxicological results indicate that while acute toxicity in wild mammals is unlikely under worst-case exposure conditions, repeated exposure may have adverse health effects (Rogers et al., 2002; Quagraine et al., 2005). NAs as surfactants penetrate more easily to cell wall and, thus NAs have reported toxic to microorganisms, algae, and invertebrates (Mackinnon et al., 1986; Rogers et al., 2002; Leung et al., 2003; Quagraine et al., 2005).

2.6 Remediation Methods for Naphthenic Acids Contaminated Water

Although, various practices and approaches have been employed to remediate the NAs contaminated water. Current methods include microfiltration, ultrafiltration, micellar-enhanced ultrafiltration (MEUF), chemical treatment, photolysis, biodegradation and advance oxidation to remediate oil sands process water (OSPW). Among the above-mentioned methods, bioremediation is considered to be best for treatment of NAs contaminated water, because of economic benefits and high public acceptance (Scott et al., 2008). Various methods with their limitations and strengths are discussed here.

2.6.1 Chemical Treatment

Under this method, the chemical reaction is used to treat the contaminant, they are fixed, destroyed or neutralized; therefore more recalcitrant organic contaminants can be easily destroyed or converted to harmful ones. The problem is when contaminants do not completely destroy. In this case, the reagent itself may cause damage to water. At the same time, an additional secondary treatment is needed (Evans, 2003; Gan *et al*., 2009). Various forms of chemical methods have been employed to treat NAs contaminated water and are effective for treatment of more persistent and recalcitrant fractions of NAs.

Chemical oxidation process degrades the pollutants; using common oxidants used in wastewater treatment include chlorine (Cl_2), hydrogen peroxide (H_2O_2), ozone (O_3), and permanganate (MnO_4^-) (Singer et al., 1999; Allen, 2008). Another effective approach includes catalytic decarboxylation, using a metal oxide catalyst (MgO) which converts NAs into CO_2 efficiently, thus lowering total acidity. The reaction takes place at 150-250 ^{0}C, but the reaction mechanism is complex (Zhang et al., 2005) and is not clearly understood. The various other reaction intermediates formed can cause corrosion to the pipelines.

Ozonation and advance oxidation processes are popular for treatment of drinking water and industrial wastewaters. Ozonation has also been investigated for treatment of NAs contaminated with water. Ozonation was found to oxidize NAs by 95% and to reduce the OSPW acute toxicity to *Vibrio fischeri* after 130 min of treatment (Scott et al., 2008). Estrada et al. (2011) also that the ozonation of commercial NAs and OSPW, NAs with more rings and more carbon (and more H atoms) were depleted most rapidly in the process.

Thus, Scott et al. (2008) suggested that ozonation preferentially degraded the most biopersistent OSPW NA fraction, and that ozonation is complementary to the biodegradation capacity of microbial populations in OSPW. Ozonation has been applied to treat wastewater; however, high ozone doses are usually required, incurring appreciable costs (Beltran, 2004; El-din et al., 2011) and thus decreasing the economic feasibility of the treatment.

Apart from ozonation, photochemical process or photocatalysis also hold promising alternative to wastewater treatment (Doll et al., 2005). In this method, pollutants are oxidized by the radicals produced through photo-excitation of a valence electron on the surface of the catalyst (Bahnemann, 2004). The most commonly used catalyst is titanium oxide (TiO_2), because of its activity, stability in aqueous solution and non-toxic characteristics (Hsien et al., 2000; Mishra, 2009).

The suitable ultraviolet wavelengths for most wastewater treatments are the UV-B (280 to 315 nm) and UV-C (200 to 280 nm) sub ranges (Protosawicki et al. 2002), and UV_{254} radiation has the most potential for remediation of naphthenic acids (Dutta et al., 2000; McMartin et al., 2004). Normally, photocatalyst particles are either immersed in the contaminated water as slurry or fixed as a bed in a proper reactor (Devipriya et al., 2005).

Various authors have reported the successful photocatalytic oxidation of aliphatic and aromatic carboxylic acids, including naphthenic acids. McMartin (2003) reported that the use of photolysis as a pre-treatment of NAs prior to the biological treatment increases the bioavailability of NAs to microorganisms, which is beneficial for the latter biodegradation stage. Headley et al. (2009) studied the photocatalytic oxidation of commercial Fluka NAs mixture and a model NA (4-methyl-cyclohexane acetic acid, 4MCHAA) and reported that under natural sunlight irradiation over the TiO_2 suspension, 75% of NAs mixture (64 mg/L) and 100% of 4MCHAA (1.5 mg/L) were degraded within 8 hrs. However, no degradation occurred under dark conditions, regardless of presence or absence of TiO_2.

One of the key disadvantages of photocatalysis treatment is that, degradation rates are strongly dependent on the efficiency of adsorption of pollutants on the catalysts. Since the penetration of highly energetic radiation wavelengths (such as those in the UV portion of the optical spectrum) is most often limited to the top several millimetres of surface waters (depending on turbidity, colour, and other physical properties) natural photolysis of

naphthenic acids is likely to be severely limited (Quagraine et al., 2005). Thus, these factors increase the cost of wastewater treatment, and leading to hamper the commercialization of the process for treatment of OSPW (Bahnemann, 2004; Allen, 2008).

2.6.2 Physical Treatment

Adsorbents are used to remove a wide variety of pollutants associated with oilfield produced waters, especially organic carbon compounds, oil and grease, and heavy metals (Allen, 2008). Adsorbents can be activated carbon, natural organic matter, zeolites, clays and synthetic polymers.

Hansen and Davies (1994) reported the removal of 95-100% for naphthenic acids from OSPW using activated carbon as adsorbent. To improve the adsorption capacity, Marr et al. (1996) used activated carbon with the acidification for removal of naphthenic acids from OSPW and removal rate was 80-100%. Problems associated with these types of treatment are low adsorption capacity, cleaning and regeneration costs of the adsorbents.

Micro and ultrafiltration are pressure-driven membrane processes that reject particles as small as 0.1 µm and 0.01 µm respectively (Allen, 2008). Farnand and Sawatzky (1985) used synthetic polymer and ceramic membrane, lab and pilot scale studies using membrane to treat produced waters have shown over 90% oil rejection with permeate concentrations of less than 20 ppm. But on wider scale, problems such as fouling and membrane durability could occur (Allen, 2008).

Nanofiltration has the potential for partial demineralization, softening, and removal of soluble organic compounds from produced water as it can reject divalent ions, dissolved organic matters, pesticides and other macromolecules (Allen, 2008). Peng et al. (2004) reported the removal rate of 95% of NAs in OSPW using nanofiltration (polyamide). Although nanofiltration has proven to be effective, but membrane fouling and membrane replacement costs has limited the use of technology.

2.6.3 Bioremediation

The term "bioremediation" is a grouping of technologies that uses microbiota (typically, heterotrophic fungi and bacteria) to degrade or transform hazardous contaminants to materials such as carbon dioxide, water, inorganic salts, microbial biomass, and other by-products that may be less hazardous than the parent materials. The process has ability to destroy wide range of organic compounds in a reasonable time. The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site (Vidali, 2001). For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products.

With the rapid increase in demand of crude oil from oil sands, there is concomitant expansion of the production of associated wastewaters containing the NAs. Bioremediation of such waste is considered to be highly reliable treatment, because of economic benefits and high public acceptance. Nowadays, different bioremediation techniques are applied to treat contaminants. For instance, they are carried out either on site or out of it. Based on the location where the bioremediation process occurs, bioremediation processes can be classified as follows:

- ***In situ***, this refers to bioremediation processes that occur below the ground surface, where the contaminated zone becomes the bioreactor.
- ***Ex situ***, this involves the use of aboveground bioreactors to treat contaminated soil (in slurry bioreactors) or groundwater (in conventional suspended or attached growth bioreactors) that has been extracted from the contaminated site.

The control and optimization of bioremediation processes is a complex task and depends on many factors. These factors include (Vidali, 2001):

- The existence of a microbial population capable of degrading the pollutants.
- Environment factors such as temperature, pH, the presence of oxygen or other electron acceptors and nutrients to ensure the maximal growth and activity of microbes.
- Toxic or inhibitory effects of the contaminants and their degradation products.
- The bioavailability of contaminants for the microbial population.

It is not hard to enrich and/or isolate a microbial population which is capable to degrade the contaminants in the environment due to the adaptation. A variety of indigenous microbes (bacteria) from tailing ponds, as well non-indigenous microbial (bacteria) species have been investigated for their potential to degrade NAs from sources like OSPW, commercial and the model compounds (Clemente etal., 2004; Scott et al., 2005; Del Rio et al., 2006; Birkyukova et al., 2007). Environmental factors including the amount of oxygen and nutrients for the microbes, the temperature and pH in the system can often be regulated to the optimal values in *ex situ* bioremediation processes. In aerobic conditions, microorganisms use available atmospheric oxygen as an electron acceptor to function. Under anaerobic conditions, no oxygen is present and biological activity is supported by the presence of other electron acceptors.

2.6.3.1 Aerobic biodegradation of NAs

The aerobic biodegradation of NAs has been documented in various studies. Predominantly various bacterial *Pseudomonas, Alcaligens, Acninetobacter, Kurthia, and Xanthomonas* species in the tailing ponds were able to readily degrade simple model NAs and NAs from commercial sources, but degradation with NAs from tailing ponds was less efficient (Clemente et al., 2004; Quagraine et al., 2005; Paslawski et al., 2008; Quensel et al., 2011). Scott et al., (2005) reported that microbial degradation of commercially available NAs was complete in 14days, in comparison to NAs in tailing waters which were still remaining after 40days.

Various factors have suggested for the degradation of NAs at slow rate. Based on the studies, there is an influence of chemical structure on biodegrability of NAs (Whitby, 2010). Generally the more persistent NAs have high molecular weight, contain multiple alkyl chains and methyl substituted cycloalkane rings (Smith et al., 2008; Paslawski et al., 2008; Johnson et al., 2010). Thus, linear and lower molecular weights NAs in oils sands are removed more rapidly by biodegradation (Quagraine et al., 2005; Paslawski et al., 2008).

Other influencing factors are temperature, DOC, reactor configuration and pH, For example Tanapat (2001) studied three model NAs (*cis*- and *trans*- isomers of 4-MCHAA, *trans* 4-MCHCA, and 3-MHCCA) indicating that temperature had the most significant effect on biodegradation kinetics with an observed ten-fold increase in the first order rate constant

between 10 ^{0}C and 30 ^{0}C. Quail et al. (1991) demonstrated that the rate of biodegradation can be greatly improved by treating the pollutants using optimum environmental conditions and a better designed and controlled bioreactor. Paslawski et al. (2008) investigated the enhancement of biodegradation of model NAs (*trans*- 4-methyl-1-cyclohexane carboxylic acid) and reported that the biodegradation rate can be significantly improved by varying the environmental conditions (temperature and pH) and reactor configuration. Another study by Huang & Nemati (2011) involving three model NAs (*cis* and *trans*- isomer 4-MCHAA, *trans*- 4MCHCA) demonstrated the effect of reactor configuration, increasing the maximum specific biodegradation rate of model NAs in circulating packed-bed bioreactor by 4-times than rate reported by Paslawski et al. (2008) in the packed bed bioreactor.

These investigations have led to the identification of number of controlling parameters for biodegradation of NAs and their recalcitrant behavior in OSPW. Further, these studies have been the references for anaerobic biodegradation.

2.6.3.2 Anaerobic biodegradation of NAs

The decomposition of organic compounds by populations of strictly anaerobic bacteria has received a great deal of attention over the past decade and as a result the importance of anaerobic decomposition in the global carbon cycle is becoming apparent. The successful application of anaerobic technology to the treatment of industrial wastewaters is critically dependent on the development and use of high rate anaerobic bioreactors. Various advantages of anaerobic treatment in comparison to aerobic treatment are (Bajpai and Bajpai, 1999; Seghezzo et al., 2001):

- ***High efficiency***. Good removal efficiency can be achieved in the system, even at high loading rates and low temperatures.
- ***Simplicity***. The construction and operation of these reactors is relatively simple.
- ***Flexibility***. Anaerobic treatment can easily be applied on either a very large or a very small scale.
- ***Low space requirements***. When high loading rates are accommodated, the area needed for the reactor is small.
- ***Low energy consumption***. As far as no heating of the influent is needed to reach the working temperature and all plant operations can be done by gravity flow, the energy

consumption of the reactor is almost negligible. Moreover, energy is produced during the process in the form of methane.

- ***Low sludge production***. The sludge production is low, when compared to aerobic methods, due to the slow growth rates of anaerobic bacteria. The sludge is well stabilized for final disposal and has good dewatering characteristics. It can be preserved for long periods of time without a significant reduction of activity, allowing its use as inoculums for the start-up of new reactors.
- ***Low nutrients and chemicals requirement***. Especially in the case of sewage, an adequate and stable pH can be maintained without the addition of chemicals. Macronutrients (nitrogen and phosphorus) and micronutrients are also available in sewage, while toxic compounds are absent.

Anoxic ecosystems are created when oxygen consumption exceeds its supply, e.g. in soils with impeded drainage, stagnant water, municipal landfills, sewage treatment digesters, industrial plants that produce methane from organic waste, and finally sediments of the oceans and other natural bodies of water. Addition of oxygen to the subsurface is difficult due to its low solubility, and therefore, metabolic fate of organic compounds depends on the inorganic electron acceptors such as (NO_3^-, Fe^{3+}, SO_4^{2-} and CO_2) available to subsurface microorganisms. Nitrate is a preferable alternative to oxygen as its energy yield is close to oxygen, it is highly water soluble harmless product (660 g/L), and it does not precipitate oxides, is inexpensive and is non-toxic to aquifer microorganisms at concentrations below 500 mg/L (Wilson & Bouwer 1996).

Nitrate containing streams are generally removed by denitrification. Denitrification is a reduction of nitrate through the intermediates of nitrite, perhaps nitric oxide and nitrous oxide to form dinitrogen gas (Feleke & Sakakibara, 2002; Killingstad et al., 2002). The reaction proceeds as shown in Equation 1, and is carried out by denitrifying bacteria. The electrons required for reduction of nitrate and other intermediates are supplied through oxidation of some suitable organic compounds such as acetate, starch, glucose, cellulosic materials such as bark, wood chip, and organic containing wastewater.

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad (1)$$

Many bacteria are capable of growing under denitrifying condition, some are heterotrophs, autotrophs and one group is photosynthetic. A potential of denitrification exists in every habitats, but from the frequency of isolation *Psuedomonas* and *Alcaligens* groups are of greatest significance (Knowles, 1982). Anaerobic bacteria have been associated with the tailing ponds of oil sands (Holowenko et al., 2001; Quagraine et al., 2005). However much less is known about the ability and mechanisms by which these can utilize NAs for degradation.

As most of tailing ponds are anaerobic (Clemente et al., 2005), Holowenko et al. (2001) studied the effect of NAs on methanogenesis. Microorganisms from oil sands containing methanogens were grown under anaerobic conditions using commercial NAs, NAs from OSPW and surrogate or model NAs. Neither commercial NAs, nor NAs from OSPW could stimulate the production of methane by microorganisms either from tailing ponds or domestic sewage sludge in the experiments. The methane was produced by surrogate or model NAs (3-cyclohexylpropanoic acid and 4-cyclohexylbutanoic acid) at high concentration (200 mg/L) in another set of experiment using only microbes from tailing ponds. As well, a high concentration of methane was also measured, when surrogates were added to domestic sludge and these model NAs underwent mineralization.

A recent study indicated the existence of anaerobic bacteria in tailing pond waters; these included anaerobic heterotrophic bacteria, sulphate reducing bacteria, and methanogens (Quagraine et al., 2005). The literature suggests, that to stimulate bioremediation various inorganic microbial nutrients are used such as nitrate or nitrogen gas, phosphorous as orthophosphate. Studies have been conducted in the laboratory and in the field showing that the presence of phosphorous enhances bacterial growth and ensuring rapid bacterial degradation of NAs and other inorganic pollutants (Lai et al., 1996). However, it is the macronutrients (N and P) that are of major concern in stimulating bioremediation and thus high concentrations of electron acceptors are required to enhance the anaerobic microbes such as nitrate-reducing bacteria, sulphate-reducing bacteria, and iron-reducing bacteria (Quagraine et al., 2005).

Chapter 3
RESEARCH OBJECTIVE

As mentioned in literature review, most of tailing ponds are anaerobic (Clemente et al., 2005) and there have been limited number of studies on anaerobic degradation of NAs. Anaerobic biodegradation of model compounds under denitrifying conditions has not been investigated and thus, it became the knowledge gap. Octanoic acid is a linear model NA and commonly found in produced water from oil sands processing (Yen et al., 2004). Although these low molecular weight compounds are known to be biodegradable, but at the same time, they are toxic to aquatic environment (Paslawski et al., 2008). Octanoic acid acted as carbon source for the denitrification in this study and culture already developed in the lab was acclimatized under anaerobic conditions was utilized. To best of our knowledge, this is the first study to investigate anaerobic biodegradation of linear model NA under denitrifying conditions

The specific objectives of this work are:

1. Anerobic biodegradation studies of model NA, octanoic acid under denitrifying conditions in batch bioreactors.
    - To study the effect of initial concentration of octanoic acid (100, 250, 500 mg/L) under varying concentration of nitrate 10 mM (620 mg/L) and 20 mM (1240 mg/L) on biodegradation kinetics and nitrate reduction rate.
    - To study the effect of temperature (10, 15, 20, 25, 30, 35 $^{\circ}$C) on biodegradation rate of octanoic acid (250 mg/L) with the nitrate concentration 10 mM (620 mg/L).

2. To study biodegradation of octanoic acid under denitrifying conditions in a continuous bioreactor at nitrate concentration of 15mM (930 mg/L). It involves:
    - Effect of residence time.
    - Effect of loading rate on removal rate.

3. Analysis of kinetic data obtained from the above investigations.

Chapter 4
MATERIAL AND METHODS

4.1 Selection of Model Naphthenic Acids

Based on the existing literature, various substrates (NAs) have been used for research and investigation for studying biodegradation and these falls into three categories: surrogate or model naphthenic acids which are individual surrogate (pure) naphthenic acid fitting the formula $C_nH_{2n+Z}O_2$, commercially available mixture of NAs (i.e. Fluka or Kodak), and NAs extracted from the oil sands tailing ponds water (Clemente et al., 2005).

Only few studies have focused on biodegradation of individual compounds of commercially available and more specifically linear and single-ringed compounds (Peng et al., 2002; Tanapat et al., 2001; Paslawski et al., 2009). To conduct the experiment, a linear model NA, octanoic acid was selected to investigate the anaerobic biodegradation kinetics in the batch and continuous reactors. Octanoic acid belongs to naphthenic acids which are commonly found in produced water (Yen et al., 2004; Quagraine et al., 2005; Deriszadeh et al., 2008). Rowland et al., (2011) also demonstrated that OSPW and commercial mixtures contain acyclic acids (octanoic acid).

Octanoic acid (CAS NO. 124-07-2) purchased from Sigma-Aldrich Co. (~97 % purity). At the room temperature, octanoic acid appears physically yellow oily liquid. The molecular formula for this compound is $CH_3(CH_2)_6COOH$ with molecular weight of 144.21 Daltons and fits the formula $C_nH_{2n+Z}O_2$ with Z=0, represents linear NA. The molecular structure for the octanoic acid is shown below in Figure 4.1.

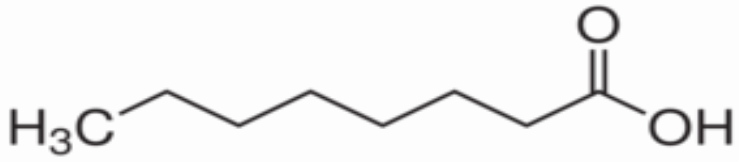


Figure 4.1: Molecular structure of octanoic acid (Sigma Aldrich Co., 2009)

4.2 Microbial Cultures and Medium

The microbial culture utilized during this study was isolated from the soil of an industrial sites contaminated with hydrocarbons using commercially prepared NAs (Fluka, Sigma-Aldrich, CAS No. 1338-24-5) as substrate in Mc-Kinney's medium (Paslawski et al., 2008).

The culture was capable of degrading trans-4MHCA and studies demonstrated consortium comprises as mixed culture, with dominant bacterial species being *Pseudomonas aerugonisa* and *Variovax paradoxus* (Paslawski et al., 2008). The same culture was used as inoculums for biodegradation of octanoic acid under denitrifying conditions and nitrate as electron acceptor.

4.2.1 Medium

Mc-Kinney's modified media containing non-growth rate limiting concentrations of all required nutrients was used for growth and maintenance of microbial consortium and biodegradation studies of octanoic acid. The medium composition was selected based on previous studies (Paslawski et al., 2008; Hill, 1974). The medium was prepared in 2 L batches of reverse osmosis (RO) water and had the following composition: KH_2PO_4 (840 mg/L); K_2HPO_4 (750 mg/L); $(NH_4)_2SO_4$ (474 mg/L); NaCl (60 mg/L); $CaCl_2$ (60 mg/L); $MgSO_4 \cdot 7H_2O$ (60 mg/L); Fe $(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (20 mg/L). Trace mineral medium was added to the macronutrients at a concentration of 0.1% on volumetric basis. The trace mineral medium comprised of: H_3BO_3 (600 mg/L); $CoCl_3$ (400 mg/L); $ZnSO_4 \cdot 7H_2O$ (200 mg/L); $MnCl_2$ (60 mg/L); $NaMoO_4 \cdot 2H_2O$ (60 mg/L); $NiCl_2$ (40 mg/L); and $CuCl_2$ (20 mg/L). The medium was sterilized at 121 $^{\circ}$C for 30 min prior to use. Various concentrations of nitrate as terminal electron acceptor were prepared from potassium nitrate (KNO_3).

4.2.2 Acclimatization of the culture

The culture isolated above during the earlier work had been acclimatized to the anerobic conditions. The initial concentration of octanoic acid (250 mg/L) and nitrate 10 mM (620 mg/L) was used and culture was grown in a serum bottle containing 100 ml of Mc-Kinney's media and purged with nitrogen gas to create anaerobic conditions at room temperature 23±1.5 ^{0}C. Measurement of optical density, octanoic acid concentration and nitrate was done at regular intervals. Following the complete biodegradation of the octanoic acid, this culture was used as inoculums for further experiments. Subculturing was carried out in the same way described above after 7-8 days.

4.3 Experimental Design and Start-up

4.3.1 Batch experiments

After the establishment of suitable microbial culture, batch experiments were carried out in 125 ml serum bottles each containing 100 ml of Mc-Kinney's medium, containing substrate (octanoic acid) at various concentrations of 100, 250, 500 mg/L, with an initial concentration of nitrate 10 mM (620 mg/L) and 20 mM (1240 mg/L). Each serum bottle was purged with nitrogen gas for 5 minutes to create anaerobic conditions and bottles were sealed with rubber septum and aluminium cap. All bottles were kept on shaker for dissolution of octanoic acid, overnight at room temperature (23±1.5 °C) before inoculation. Each bottle was inoculated with the 7-day old culture (10% on volume basis) grown on 250 mg/L octanoic acid and 10mM (620 mg/L) nitrate and was maintained at room temperature (23±1.5 °C). The effects of various substrate concentrations were investigated to evaluate the ultimate capability of the microbes to degrade octanoic acid under anaerobic conditions. All the batch experiments were done in duplicates for reproducibility. All experiments were carried out simultaneously, with substrate concentrations of 100, 250 and 500 mg/L having 10 mM nitrate and further increased to 20 mM nitrate for octanoic acid 500 mg/L, so that limiting nutrient would not be nitrate. Control experiments were conducted under similar conditions without inoculation with lowest and highest substrate concentrations (100 and 500 mg/L) having nitrate concentration of 10 mM (620 mg/L) and 20mM (1240 mg/L).

To assess the effect of temperature on biodegradation of octanoic acid under anerobic denitrifying conditions, experiments were conducted progressively by lowering from 23 °C to (20, 15, 10 °C) from and similarly by increasing it to higher temperature (30, 35 °C) ranges. The initial substrate concentration for temperature experiments was 250 mg/L and 10 mM (620 mg/L) nitrate. All the experiments were performed in temperature controlled chambers to maintain desired temperature.

In all cases, sampling was done at regular intervals, before sampling the serum bottle was gently shaken and about 1.5 ml of sample was taken using glass syringe. Sampling frequency was increased at exponential stage and decreased at lag and stationary phase. Measurement of optical density for biomass, octanoic acid, nitrate and nitrite concentration were done in every sample. The samples were filtered through 0.22 µm nylon membrane filters using a stainless steel cartridge to remove biomass prior to analyzing the substrate and nitrate ion concentration.

4.3.2 Experiments in Continuous Stirred Tank Bioreactor

The continuous stirred tank bioreactor (CSTR) was set up for removal of octanoic acid under denitrifying conditions. The reactor vessel was constructed of glass, with a working volume of 200ml as shown in Figure 4.1. Initially, bioreactor was operated batch wise, at room temperature 23±1.5 $^{\circ}$C with an initial concentration of 250 mg/L and 10 mM nitrate and purged with nitrogen gas to create anaerobic conditions. The reactor was inoculated with from 7-day old culture 10% (v/v) and magnetic stirrer was used to achieve mixing and maintain biomass and substrate in suspension. The bioreactor was switched to continuous mode when octanoic acid and nitrate were completely utilized. To feed the reactor with continuous supply of medium with octanoic acid (250 mg/L) and nitrate 10 mM (620 mg/L) peristaltic pump was used.

Prior to pumping the feed, was purged with nitrogen gas and even at regular intervals the reactor was also purged to create anaerobic conditions. The initial flow rate was 10 mL/hr and effluent was removed by the overflow tube. During the operation of the reactor, samples were taken from the reactor and it was noticed nitrate was limiting and was increased to 15 mM (930 mg/L). At each flow rate, sufficient time was given to establish the steady state which was assumed when residual concentrations of octanoic acid and nitrate were relatively constant in bioreactor (change by 10%). The samples were taken from rubber septum located in the middle of bioreactor, for determining biomass (OD), residual octanoic acid, and nitrate and nitrite concentrations. The flow rate was increased incrementally from 10 mL/hr to 150 mL/hr till washout conditions were reached. The peristaltic pump was calibrated before use for various flow rates and verified by measuring the effluent volume after some interval. Applied dilution rates were in the range of 0.05 to 0.75 h^{-1}.

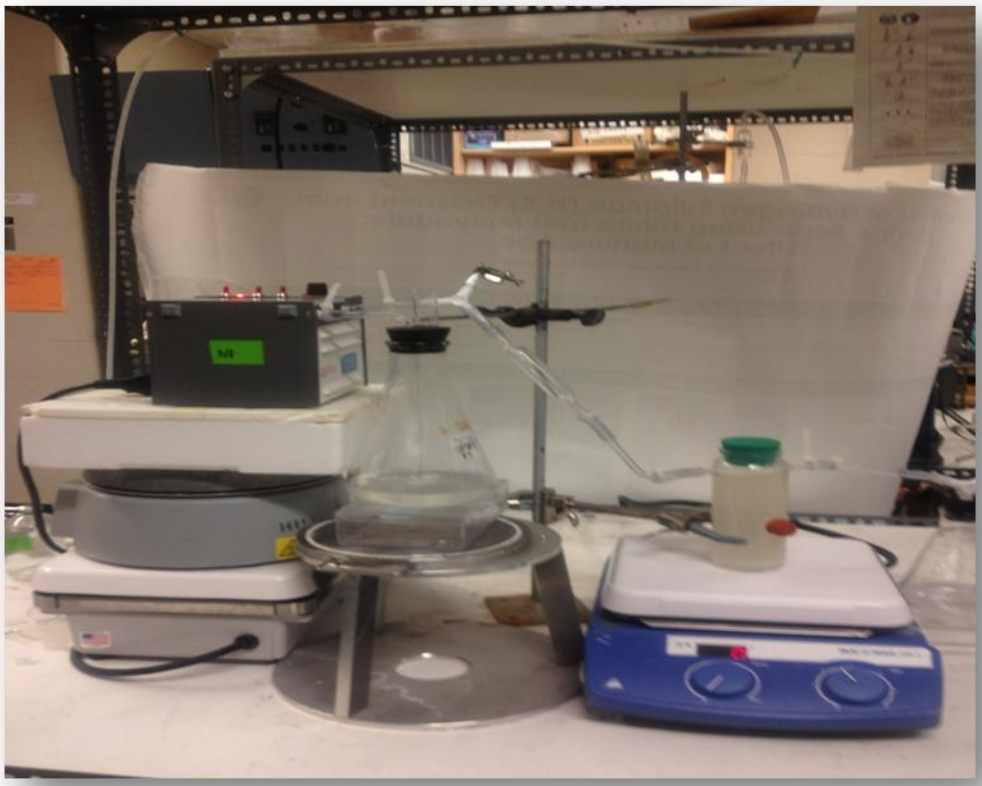


Figure 4.2 Continuous stirred tank bioreactor set-up for degradation of octanoic acid under denitrifying conditions

4.4 Analytical Methods

4.4.1 Measurement of Biomass Concentration

The concentration of free biomass was determined by direct measurement of the optical density (OD) of samples collected from batch and continuous bioreactors using the glass needles. The OD was measured at wavelength of 620 nm using spectrophotometer (Shuler, 2002). An UV-visible spectrophotometer (Mini Shimazdu, Model 1240) was used for determining the OD. The OD was then related to dry weight using the calibration curve developed in these experiments. The standard curve is shown in Appendix as Figure A.1

4.4.2 Measurement of Octanoic Acid Concentration

The assessment of naphthenic acids is limited by analytical techniques because of the concentrations and types of acids present in these complex oil sands mixtures. There is currently no method or technique that quantifies or separates individual acids of the mixture. Thus, the most of techniques rely on the treating them as group or sub-group,

based on the carbon or Z numbers (Clemente et al., 2005). Various techniques have been used and these includes, high-performance liquid chromatography (HPLC), fourier transform infrared (FTIR) spectroscopy, negative ion electrospray ionization-mass spectrometry (ESI-MS), gas chromatography with a flame ionization detector (GC-FID), gas chromatograph-mass spectrometry (GC-MS), liquid secondary ion mass spectrometry (LSI-MS), electrospray ionization (ESI), and quantitative quadrupole time of flight –MS (QTOF_MS) (Paslawski et al., 2008; Bataineh et al., 2006; Clemente et al., 2005; Barrow et al.,2004). All of the above methods have unique strengths as well limitations.

To analyse the model compound octanoic acid, we followed the method developed by Paslawski et al. (2008) using gas chromatography with a flame ionization detector (GC-FID) to accurately determine NAs concentrations in water. The method was adopted due to its simplicity in analysing the model NAs compound. The analytical equipment used in this study was a Varian- 430 gas chromatograph, where helium served as carrier and makeup gas and hydrogen and air were used as combustion gas in the FID. A HP-INNOWAX high resolution gas chromatography column (19091N-133) was used. The column had the following specifications: length of 30 m, inside diameter of 0.250 mm, and film thickness of 0.25 μm. The operating conditions for the system were as follows:

- H_2 flow rate: 30 ml/min
- He flow rate: 29 ml/min
- Air flow rate: 300 ml/min
- Injector split ratio: 1to10
- Column oven initial temperature: 90 °C
- Injector temperature: 220 °C
- Detector temperature: 250 °C
- Column oven temperature program: 90 °C ramped to 210 °C at a rate of 40 °C/min

A linear calibration curve was developed to convert gas chromatography readings to the actual octanoic acid concentrations (mg/L) in the sample. To develop the calibration curve, the candidate NA compound was dissolved into the sterile McKinney's modified medium. This solution was then diluted into five different concentration solutions, which were used as the standards. The 6 standard concentrations were 20.72, 51.8, 103.6, 207.2, 414.4 and 518 mg/L for octanoic acid. Each standard solution was injected three times and Millipore water

was run between samples to prevent the possible accumulation of the substrate in the column and ensure the accuracy of readings. The developed linear calibration curve for octanoic acid is shown through Figure B.2 in the Appendix. The retention time of octanoic acid was (3.03 min). Each unknown sample was injected three times, followed with the blank between samples and the average of three injections readings was taken to calculate the final concentration of the sample.

4.4.3 Measurement of nitrate and nitrite ion Concentrations

The concentrations of nitrate and nitrite ions were determined using a Dionex ion chromatograph (ICS-2500) with a conductivity detector (CD25A) equipped with an IonPac CG5A guard column and an IonPac CS5A analytical column. The eluent was 1.0 mM KOH and the flow rate of the eluent was set at 1.0 mL/h. The system was calibrated using standard solutions of nitrite and nitrate with concentrations of 5, 10, 20, 30, and 50 mg/L. To establish the calibration curves, standard solutions with each concentration were injected three times (injection volume = 25.0 uL). The calibration curves were quadratic for all the ions with standard deviation associated with measurements of nitrate, and nitrite are 1.49% and 0.90%.

To prepare the samples for IC analysis, liquid samples (0.1 mL), was transferred into a 1.5 mL micro-centrifuge tube filled with 0.9 mL Millipore water (10 times dilution). Samples were further diluted (overall dilution ratio of 40 folds) to ensure the concentrations of ions fell in the concentration ranges of the developed calibration curves. Diluted samples were then analyzed by IC, measuring the ion concentrations of nitrate and nitrite simultaneously.

4.4.4 Statistical analysis of results

All samples were taken periodically in this study and every set of batch experiments were conducted in duplicates. The average values of the data obtained in the repeated experiments were presented as final results. The standard deviation associated with data was calculated and presented as error bar.

For experiments conducted in the continuous bioreactors, following the establishment of steady state at each applied conditions, the bioreactor was run for two-three additional days and three additional samplings were done and the average value of these data was used as the final result. The standard deviation associated with the data was also calculated and presented as error bar. Wherever applicable, the value of regression coefficient was calculated and included as part of results.

Chapter 5
RESULTS AND DICUSSION

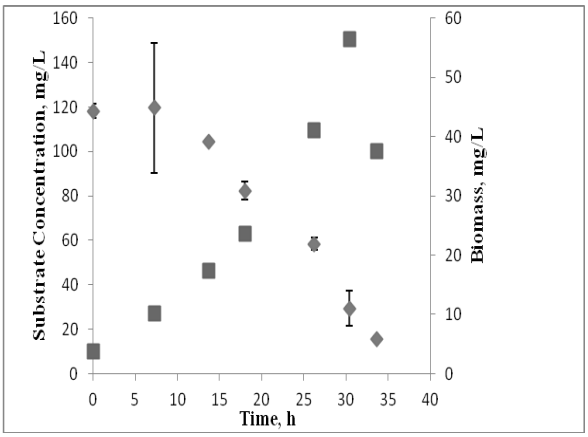
5.1 Effect of initial concentration of octanoic acid and nitrate

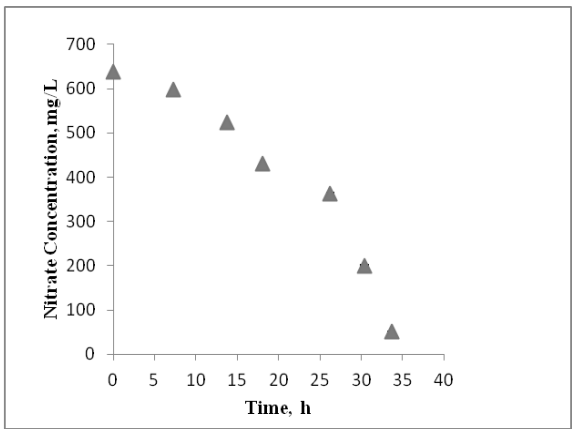
Biodegradation of octanoic acid under denitrifying conditions with different initial concentrations of octanoic acid and nitrate was observed. Figure 5.1 shows the results of microbial growth, substrate removal and nitrate reduction as function of time in batch reactors containing different initial concentrations of octanoic acid and nitrate at room temperature 23±1.5 ˚C.

Control experiments were also run in sterilized serum bottles, without inoculation at two tested concentrations 100 mg/L and 500 mg/L to show that decrease in octanoic acid took place due to bacterial activity and not by natural degradation or physiochemical process. In all biodegradation experiments a direct relationship of microbial growth and substrate and nitrate removal were observed, and specific growth rates, biodegradation rate and nitrate reduction rate were determined using exponential growth phase data.

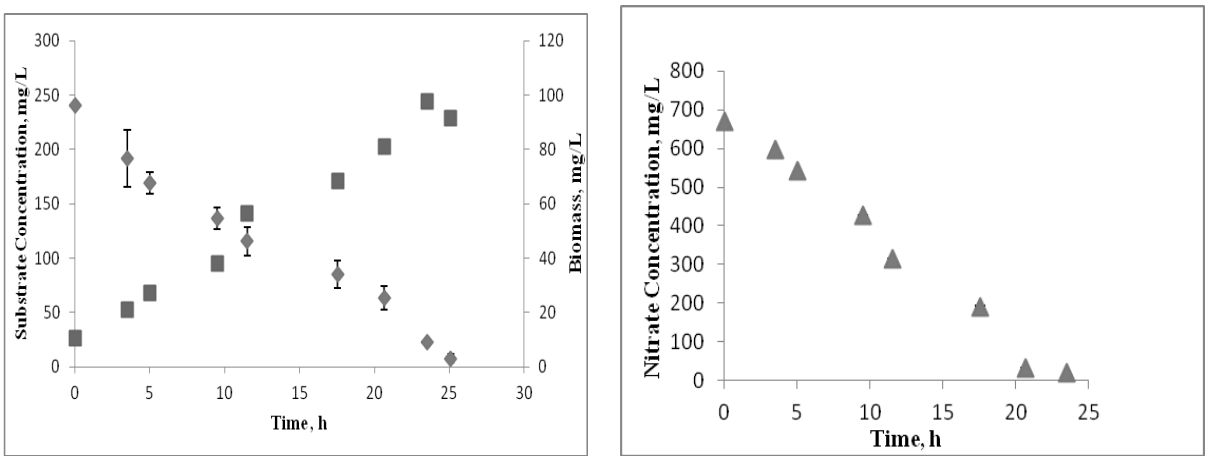
The lag phase was not observed at the concentration of octanoic acid 100 mg/L with 10 mM (620 mg/L), but on increasing the concentration of substrate the lag phase occurred and its length increased. Overall, denitrification proceeded successfully with octanoic acid as carbon source and nitrate removal was evident in all batch reactors and no nitrite production was observed.

(A)

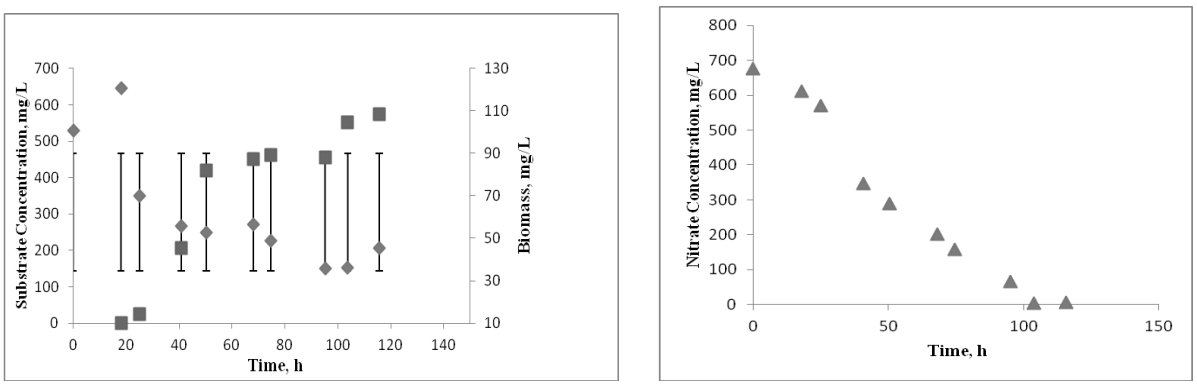




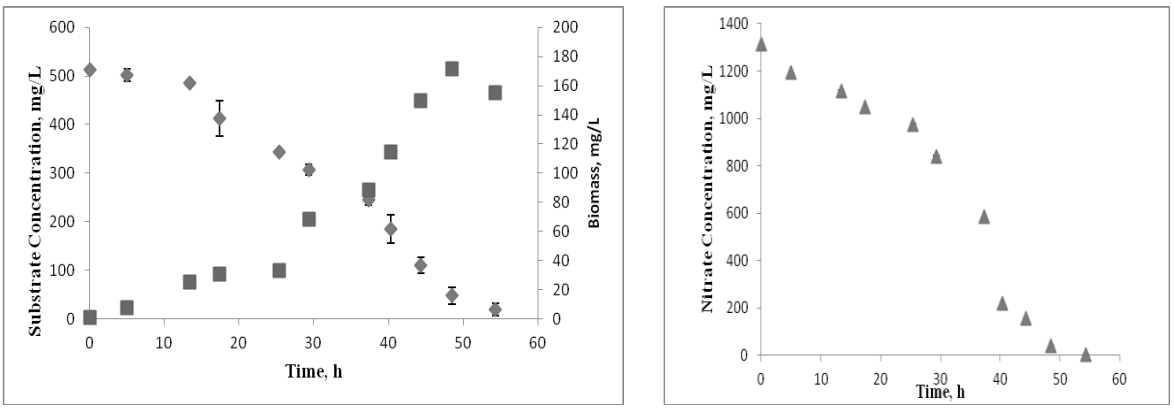
(B)



(C)



(D)



◇ Substrate concentration Δ Nitrate concentration ☐ Biomass

Figure 5.1 Substrate biodegradation, nitrate reduction and biomass growth as a function of time in batch reactors at various concentrations of octanoic acid and nitrate at temperature 23±1.5 ˚C, (A) 100 mg/l and 10 mM (620 mg/L); (B) 250 mg/L and 10 mM (620 mg/L); (C) 500 mg/L and 10 mM (620 mg/L); (D) 500 mg/L and 20 mM (1240 mg/L).

Table 5.1 shows the values of specific growth rate (calculatation in Appendix-1), biodegradation rates and nitrate reduction rate observed at different initial concentrations of octanoic acid and nitrate. The biodegradation rate and nitrate reduction rate increases with increase in initial substrate concentration, but specific growth rate increases up to a maximum of 250 mg/L octanoic acid and 10 mM (620 mg/L).

The biodegradation of octanoic acid in batch reactor with 500 mg/L and 10 mM (620 mg/L) ceased to 200 mg/L as shown in Figure 5.1 (C) due to nitrate limiting conditions and nitrate concentration was increased to 20 mM (1240 mg/L) for complete removal of octanoic acid.

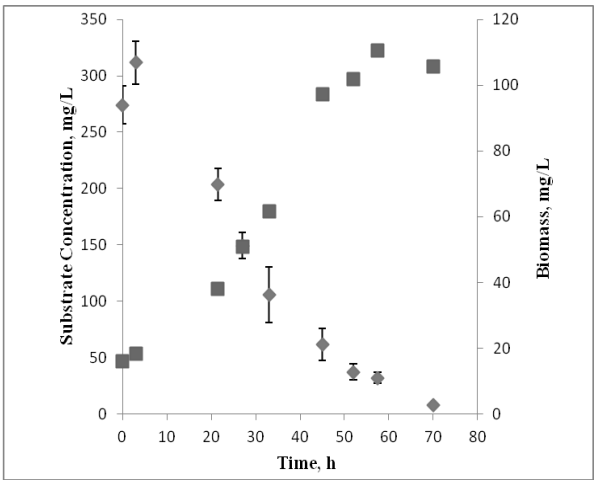
Table 5.1 Summary of specific growth rate, biodegradation rate and nitrate reduction at various initial concentrations at 23±1.5 ˚C (batch reactors).

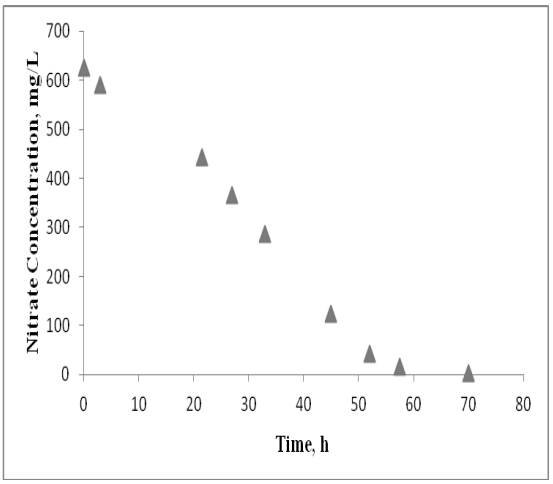
Initial Substrate Concentration mg/L	**Initial Nitrate Concentration mg/L**	**Specific Growth Rate, μ h^{-1}**	**Biodegradation Rate mg/L.h**	**Nitrate Reduction Rate mg/L.h**
100	620	0.09	4.34 (R^2=0.98)	21.51 (R^2=0.97)
250	620	0.107	7.32 (R^2=0.97)	31.461 (R^2=0.90)
500	1240	0.06	11.55 (R^2=0.96)	36.35 (R^2=0.93)

5.2 Effect of temperature on biodegradation of octanoic acid under denitrifying conditions

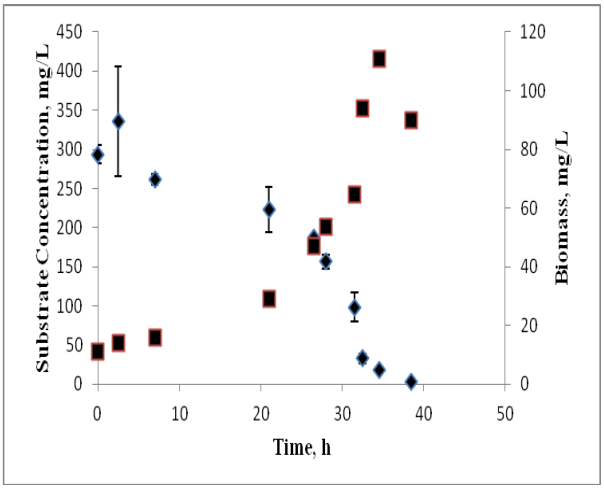
Figure 5.2 shows the results of microbial growth, biodegradation rate and nitrate reduction rate as a function of time at different temperatures 10, 15, 20, 30, 35 ˚C.

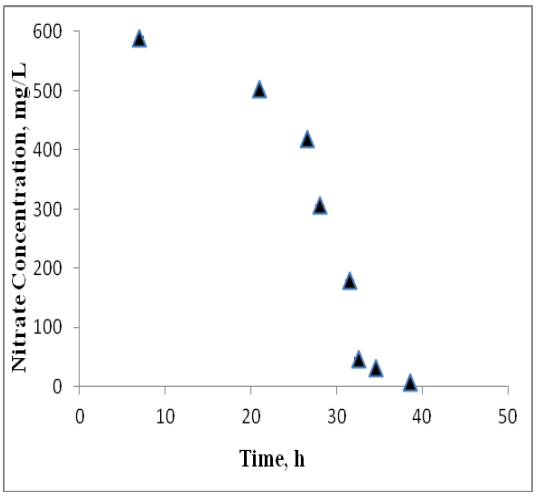
(A)


Substrate Concentration, mg/L
Biomass, mg/L
Time, h

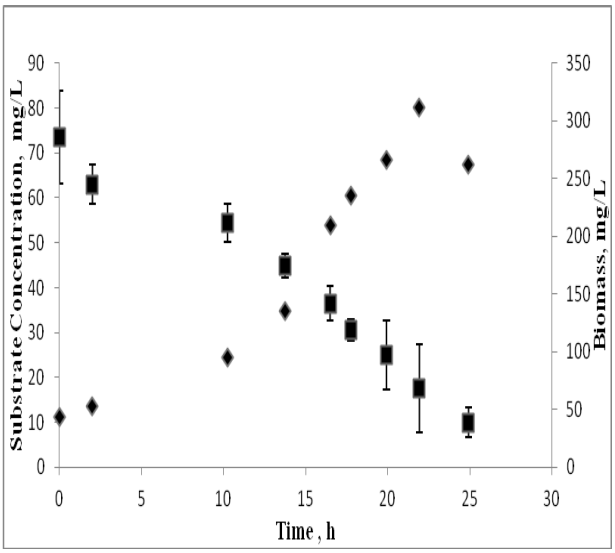

Nitrate Concentration, mg/L
Time, h

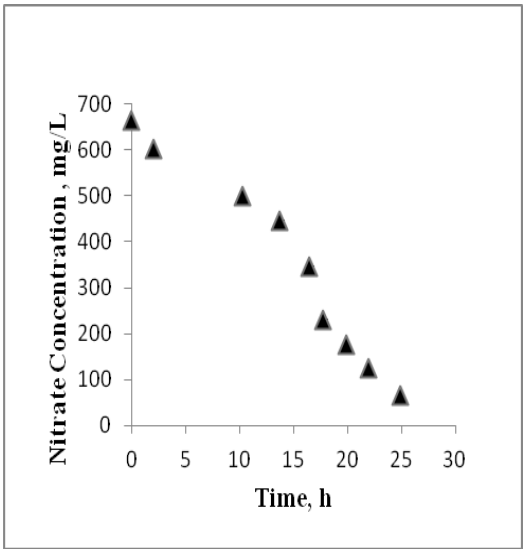
(B)


Substrate Concentration, mg/L
Biomass, mg/L
Time, h

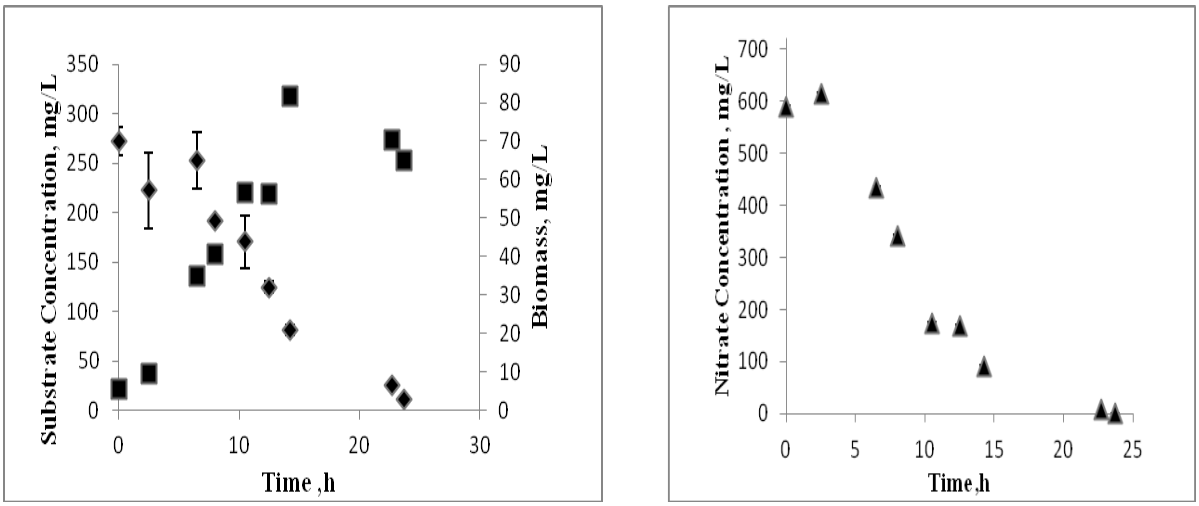

Nitrate Concentration, mg/L
Time, h

(C)

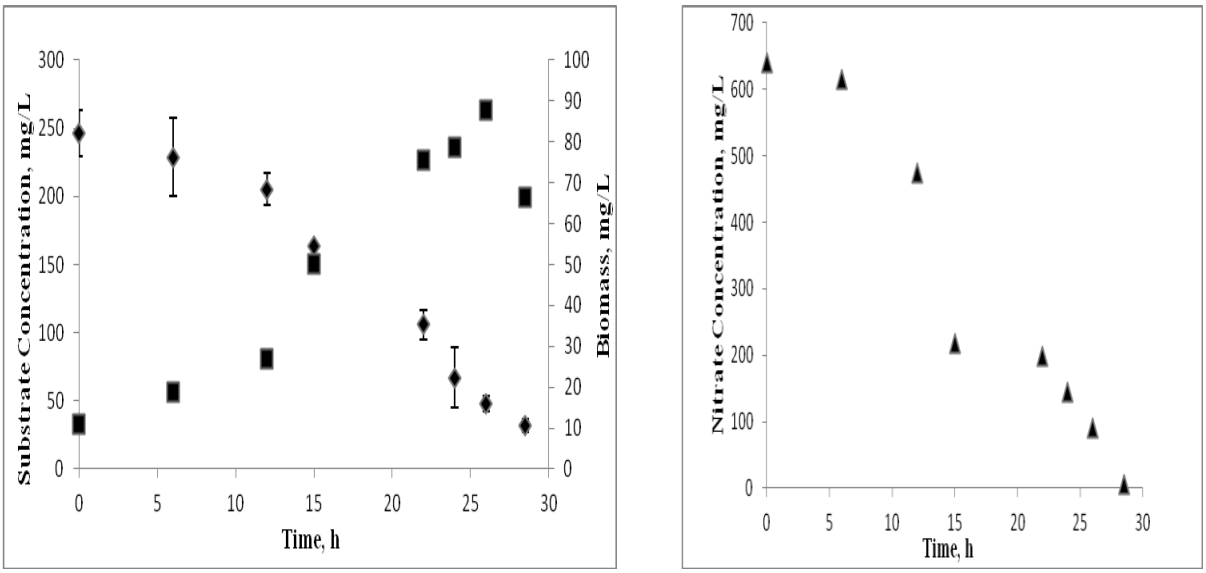

Substrate Concentration, mg/L
Biomass, mg/L
Time , h


Nitrate Concentration , mg/L
Time, h

(D)



(E)



◇ Substrate concentration Δ Nitrate concentration ☐ Biomass

Figure 5.2 Substrate biodegradation, nitrate reduction and biomass growth as function of time at different temperatures and at initial 250 mg/L octanoic acid concentration and 10 mM (620 mg/L) nitrate, (A) 10 ˚C ; (B) 15 ˚C; (C) 20 ˚C; (D) 30 ˚C; (E) 35 ˚C.

The specific growth rate, biodegradation rate and nitrate reduction rate at various temperatures for octanoic acid initial concentration of 250 mg/L and nitrate 10 mM (620 mg/L) are shown in Table 5.2.

Table 5.2 Summary of specific growth rate, biodegradation rate and nitrate reduction rate at various temperature for initial octanoic acid concentration (250 mg/L) and nitrate 10 mM (620 mg/L).

Temperature, ˚C	**Specific Growth Rate, μ h^{-1}**	**Biodegradation Rate mg/L.h**	**Nitrate Reduction Rate mg/L.h**
10	0.030	4.58 (R^2 =0.93)	12.27 (R^2= 0.99)
15	0.159	16.21 (R^2=0.90)	38.18 (R^2=0.92)
20	0.090	12.96 (R^2=0.99)	34.37 (R^2=0.94)
23±1.5	0.105	7.32 (R^2=0.97)	31.461 (R^2=0.90)
30	0.102	11.50 (R^2=0.92)	28.35 (R^2=0.93)
35	0.083	10.87 (R^2=0.98)	21.71 (R^2= 0.77)

The value of specific growth rate was found to increase with increase in temperature from 10 to 15 ˚C. But didn't change significantly when temperature was increased further. The maximum biodegradation rate of octanoic acid was observed at 15 ˚C with a value of 16.21 mg/L.h and lowest at 10 ˚C with a value of 4.58 mg/L.h. Thus, the optimum temperature for biodegradation of octanoic acid under denitrifying conditions is 15 ˚C.

5.3 Biodegradation of octanoic acid in the continuous stirred tank bioreactor under denitrifying conditions

Figure 5.3 shows the steady state profiles of biomass, substrate and nitrate concentrations obtained at different dilution rates ranging from 0.05 to 0.75 h^{-1}.

It can be seen from this figure that the biomass concentration increases with increasing dilution rate from 0.05 to 0.1 h^{-1}, while octanoic acid was completely consumed with reduction of nitrate as well.

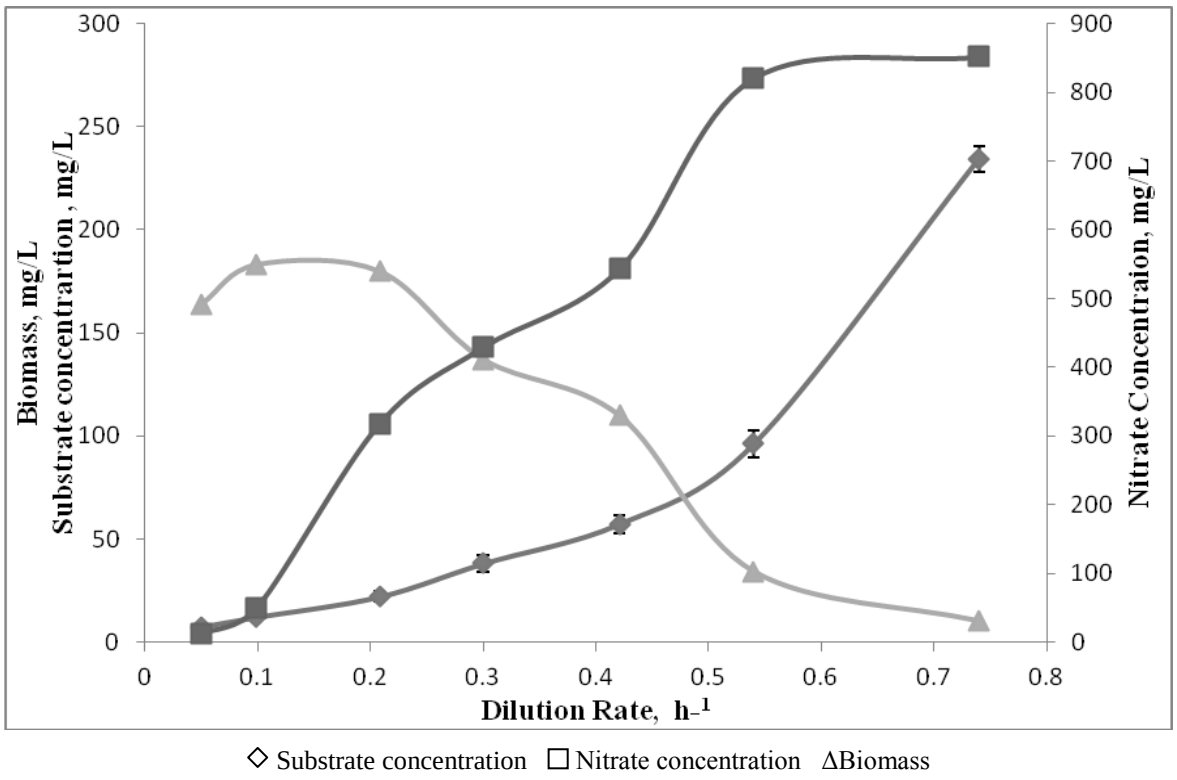
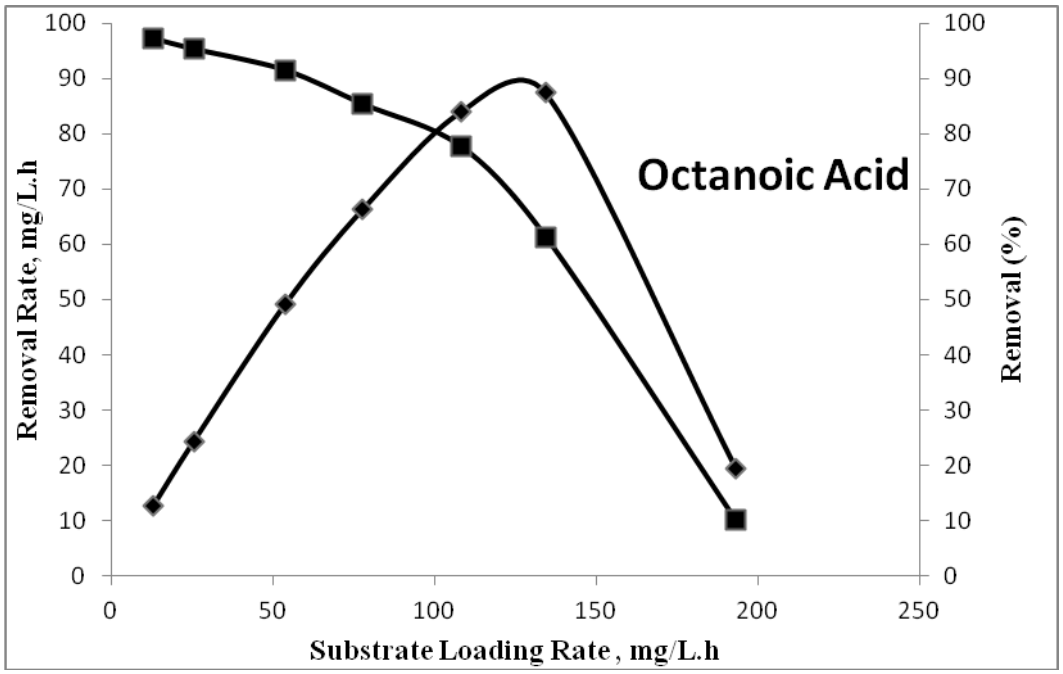


Figure 5.3 Steady state profiles of biomass, substrate and nitrate concentrations as a function of dilution rate at 250 mg/L octanoic acid and 15 mM (930 mg/L) nitrate.

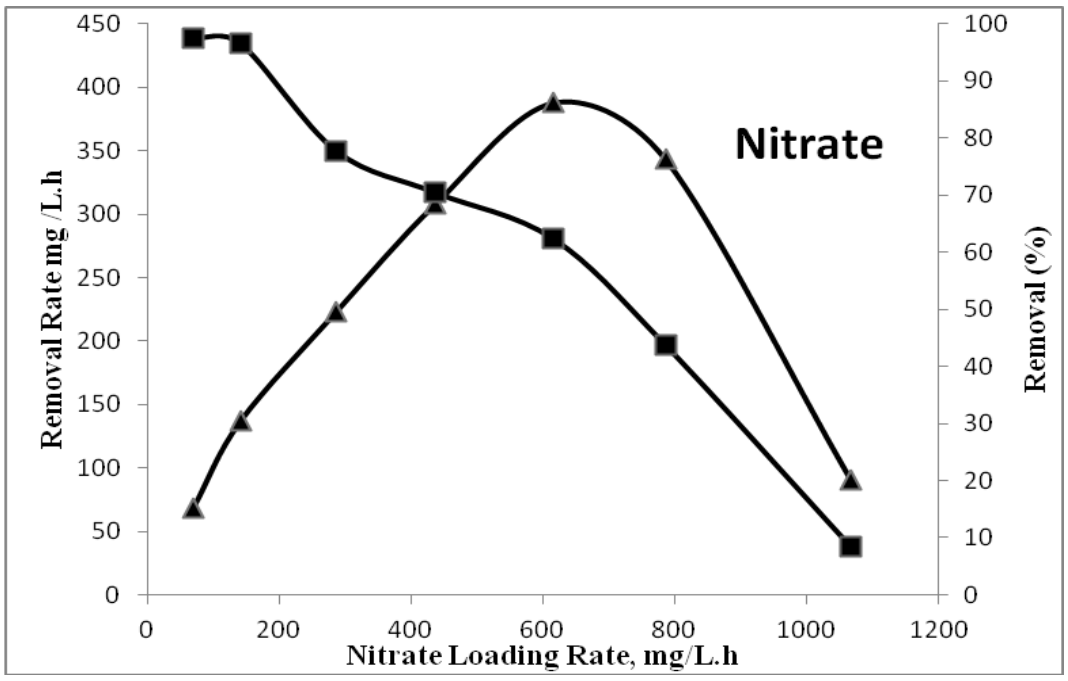
With further increase in dilution rate, the biomass decreased and the concentration of residual substrate with nitrate increased. This increase in concentration of residual substrate with nitrate approached feed concentration at dilution rate 0.75 h^{-1}. The wash out in form of decrease in concentration of biomass was observed.

The rate of removal of substrate and nitrate was obtained as function of loading rate in the CSTR operated at initial concentration of 250 mg/L octanoic acid and 15 mM (930 mg/l) nitrate as shown in Figure 5.4.

(A)



(B)



☐ Removal Percentage ◇ Substrate Loading Rate Δ Nitrate Loading Rate

Figure 5.4 Removal rate as function of loading rate in CSTR operated at an initial substrate of 250 mg/L Octanoic acid (A) and 15 mM (930 mg/L) Nitrate (B) at 23±1.5 ˚C.

The maximum removal rate of octanoic acid was observed in the range of 83.96-87.35 mg/L˙h at loading rate ranging from 108.12 to134.31 mg/L.h (residence time 2.5-2 h) and

the percentage removal was in the range of 61.3-71%. The maximum removal rate of nitrate was 388.12 mg/L.h observed at loading rate of 616.28 mg/L.h (residence time 2.5 h) with a percentage removal of 62.31%. The removal percentages for both substrate and nitrate decreased, when loading rates increased slightly from the critical value.

As mentioned earlier, there are limited studies on anaerobic degradation of naphthenic acids. So in this work anaerobic biodegradation of linear model compound octanoic acid (NA) under denitrifying conditions was successfully demonstrated in both batch and continous reactors. No study has been reported in the past on the anaerobic biodegrdation of octanoic acid under denitrifying conditions in the literature, so therfore biokinetic data could not be compared with literature data.

Chapter 6
CONCLUSIONS

The biokinetics of a linear model NA compound (octanoic acid) was studied in batch systems under denitrifying conditions in order to determine rate of biodegradation of octanoic acid and environmental factors influencing the biodegradation.

The microbial consortium was capable to degrade octanoic acid under denitrifying conditions using nitrate as electron acceptor. The maximum biodegradation was achieved at concentration of octanoic acid 500 mg/L and 20 mM (1240 mg/L) nitrate with biodegradation rate of 11.55 mg/L.h and nitrate reduction rate 36.35 mg/L.h at moderate temperatures 23±1.5 $^{\circ}$C. Biodegradation was influenced with temperature ranging between 10 and 35 $^{\circ}$C with highest biodegradation rate of 16.21 mg/L.h and nitrate reduction rate of 38.15 mg/L.h, at 15 $^{\circ}$C. Thus, the optimal temperature for microbial growth and biodegradation is 15 $^{\circ}$C. In the continuous bioreactor increase of loading rate ranging up to a value of 108.12 to 134.31 mg/L.h (residence time 2.5-2 h) caused a linear increase in biodegradation or removal rate with a maximum value ranging in 83.96-87.35 mg/L.h obtained at the higher loading rate for octanoic acid. The result for nitrate removal was observed with the same linear increase as biodegradation of octanoic acid with maximum loading rate of 616.28 mg/L.h (residence time 2 h) and removal rate 388.21 mg/L.h. Denitrification was followed in all cases of batch and continuous reactors; the nitrate removal was complete in all the cases with no nitrite production.

The results of the present study indicate that anaerobic biodegradation of octanoic acid under denitrifying conditions is influenced by temperature, concentration of nitrate and linear model NA compound, and bioreactor configuration. Though working with the model compounds than the commercial mixture or NAs from mixture in oil sands process water (OSPW) may not represent the actual case, but this may assist in understanding the mechanism of anerobic biodegradation of NAs under denitrifying conditions. As a final note, anaerobic biodegradation of model compounds under denitrifying conditions could facilitate future studies aiming at *in-situ* bioremediation of complex mixture of NAs in OSPW under anaerobic conditions with nitrate as electron-acceptor.

REFERENCES

Allen, E.W. 2008. Process water treatment in the oil sands industry: I. Target pollutants and treatment objectives. J. Environ. Eng. Sci. **7**: 123–138. doi:10.1139/S07-038.

Allen, E.W. 2008. Process water treatment in the oil sands industry:II. A review of emerging technologies. J. Environ.Eng. Sci. **7**: 499–524. doi:10.1139/S08-020.

Bahnemann, D.(2004). Photocatalytic water treatment: solar energy applications. Sol. Energy, 77:445-459. DOI10.1016/j.solener.2004.03.031.

Bajpai P.K., Bajpai P., *Environmental biotechnology for paper and pulp mill effluents*. Springer 1999.

Barrow, M. P., J. V. Headley, K. M. Peru and P. J. Derrick, "Fourier Transform Ion Cyclotron Resonance Mass Spectrometry of Principal Components in Oil sands Naphthenic Acids," J. Chromatogr. A. **1058,** 51-59 (2004).

Bataineh, M., A. C. Scott, P. M. Fedorak and J. W. Martin, "Capillary HPLCQTOF-MS for Characterizing Complex Naphthenic Acid Mixtures and their Microbial Transformation," Anal. Chem. **78,** 8351-8361 (2006).

Bessa, E., Sant'Anna, G.L., and Dezotti, J. 1999. Photocatalysis: An approach to the treatment of oil field produced waters. J. Adv. Oxid. Technol. **4**: 196–202.

Biryukova, O.V., Fedorak, P.M., Quideau S.A. (2006). Biodegradation of naphthenic acids by rhizosphere microorganisms. *Chemosphere, 67*(10), 2058-2064.

Brient, A., P.J.Wessner, M.N.Doyle (1995). Napthenic acids. In *Kirk-Othmer Encyclopaedia of Chemical Technology*, 4th ed.; Kroschwiz, J.I., Ed. JohnWiley and Sons: New York, 1017–1029.

Clemente, J.S., P.M. Fedorak(2005). A Review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids. *Chemosphere*, 60(5), 585-600.

Conrad Environmental Aquatics Technical Advisory Group (CEATAG), (1998). *Naphthenic Acids Background Information Discussion Report*, 65 pp.

Deineko, P.S., E.N.Vasil'eva, O.V. Popova, and S.T.Bashkatova (1994). Naphthenic Acids as Antiwear Additives for Jet Fuels, Chem. Technol. Fuel Oils. 30, 343-345

Devipriya, S., S.Yesodharan (2005). Photocatalytic degradation of pesticide contaminants in water. Sol energy Mat. Sol. Cell, 86:309-348. DOI: 10.1016/j.solmat.2004.07.013

Dokholyan, V.K.; A.K.Magomedov (1983). Effects of sodium naphthenate on survival and some physiological-biochemical parameters of some fishes. J. Ichthyol. *23*, 125–128.

Doll, T. E. and F.H. Frimmel (2005). Removal of selected persistent organic pollutants by heterogeneous photocatalysis in water. Catalysis Today 101:195–202.

Dutta, T.K. and S. Harayama (2000). Fate of crude oil by the combination of photooxidation and biodegradation. Environmental Science and Technology 34: 1500-1505.

Evans MG, Furlong CJ (2003). Environmental Biotechnology Theory and Applications. John & Wiley Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex PO19 8SQ, England.

Energy Resources Conservation Board (ERCB) (2011).Public Zone Oil Sands. [online]. Availablefromhttp://www.ercb.ca/portal/server.pt?open=512&objID=249&PageID=0&cached=true&mode=2 [cited April 2011].
Frank, R.A., R.Kavanagh, B.K.Burnison, G. Arsenault, J.V.Headley, K.M. Peru, G.V.Kraak and K. R. Solomon (2008). Toxicity assessment of collected fractions from an extracted naphthenic acid mixture. *Chemospere* 72: 1309-1314.

Feleke, Z., Sakakibara, Y., 2002. A bio-electrochemical reactor coupled with adsorber for the removal of nitrate and inhibitory pesticide. *Water Research* 36: 3092-3102.

Freudenhammer, H., D.W.Bahnemann, L.Bousselmi, S.U. Geissen, S.U. Ghrabi, F.Saleh, A. Si-Salah, U.Simeon, and A.Vogelpohl (1997). Detoxification and recycling of wastewater by solar-catalytic treatment. Water Sci. Technol. **35**: 149–156. doi:10.1016/S0273-1223(97)00020-6.

Fujishima, A., X. Zhang and D.A. Tryk. 2008. TiO2 photocatalysis and related surface phenomenon. *Surface Science Report* 63: 515-582.

Gan, B. A. (2006). *Environmental research at the leading edge.* New York, USA: Nova Science Publisher Inc.

Han, X. M., MacKinnon, M. D., and Martin, J. W. (2009). Estimating the in situ biodegradation of naphthenic acids in oil sands process waters by HPLC/HRMS. Chemosphere. 76, 63 – 70.

Headley, J.V. and D.W. McMartin. 2004. A review of the occurrence and fate of naphthenic acids in aquatic environments. *Journal of Environmental Science and Health: Part A* 39(8): 1989-2010

Headley, J.V., J. Du, K.M. Peru and D.W. McMartin (2009). Electrospray ionization mass spectrometry of the photodegradation of naphthenic acids mixtures irradiated with titanium dioxide. *Journal of Environmental Science and Health: Part A* 44: 591-597.

Headley, J.V., K.M. Peru, D.W. McMartin and M. Winkler. 2002a. Determination of dissolved naphthenic acids in natural waters using negative-ion electrospray mass spectrometry. *Journal of the AOAC International* 85: 182-187.

Headleyl,J.V., S.Tanapat, G.Putz, and K.M. Peru, (2002). Biodegradation kinetics of geometric lsomers of model naphthenic acids in athabasca river water. *Canadian Water Resources Journal, 27*(1)

Herman, D.C., P.M. Fedorak and J.W. Costerton. 1993. Biodegradation of cycloalkane carboxylic acids in oil sands tailings. *Canadian Journal of Microbiology* **39**: 576-580. 80
Holowenko, F.M., M.D. MacKinnon, P.M.Fedorak (2002). Characterization of naphthenic acids in oil sands wastewaters by gas chromatography–mass spectrometry. Water Res. 36, 2843–2855.

Holowenko, F.M., M.D. MacKinnon, P.M. Fedorak (2001). Naphthenic Acids and Surrogate Naphthenic Acids in Methanogenic Microcosms. Water Res. 35, 2595- 2606.
Hsien, Y. H., K.H. Wang, R.C. Ko and C.Y. Cang. 2000. Photocatalytic degradation of wastewater from manufactured fiber by titanium dioxide suspensions in aqueous solution: a feasibility study. *Water Sci. Tech.* 42:95–99.

Huang, J., Nemati, M., Hill, G., and Headley, J. (2012). Batch and continuous biodegradation of three model naphthenic acids in a circulating packed-bed bioreactor. *Journal of Hazardous Materials, 201 – 202*, 132 – 140.

Killingstad, M.V., Widdowson, M.A., Smith, R.L., 2002. Modeling enhanced in situ denitrification in groundwater. *Journal of Environmental Engineering* 128 (6): 491-504.

Kanaly, R. A. & Harayama, S. (2000). Biodegradation of high molecular weight polycyclic aromatic hydrocarbons by bacteria. *J. of Bacteriology*, 182 (8), 2059–2067.

Lai, J.W.S.; Pinto, L.J.; Kiehlmann, E.; Bendell-Young, L.I.; Moore (1996), M.M. Factors that affect the degradation of naphthenic acids in oil sands wastewater by indigenous microbial communities. Environ. Toxicol. Chem., 15,1482–1491.

Lo, C. C., B.G.Brownlee, N.J.Bunce (2003). Electrospray-Mass Spectrometric Analysis of Reference Carboxylic Acids and Athabasca Oil Sands Naphthenic Acids, Anal. Chem. **75,** 6394-6400

MacKinnon, M.D. (1989). Development of the tailings pond at Syncrude's oil sands plant: 1978–1987. AOSTRA J. Res. 5, 109–134.

Mackinnon, M., H. Boerger (1986). Description of two treatment methods for detoxifying oil sands tailings pond water. Water Pollut. Res. J. Can. *21*, 496–512.

Mandelstam, J.; McQuillen K., (1968). Biochemistry of bacterial growth. John Wiley&Sons Inc. New York.

Manonmani, H.K. D. H. Chandrashekaraiah, N. Sreedhar Reddy, C. D. Elcey, and A. A. M. Kunhi, Initials. (2000). Isolation and acclimation of a microbial consortium for improved aerobic degradation of α-hexachlorocyclohexane. *J. Agric. Food Chem*, *48*(9), 4341-4351.

McMartin, D. W., (2003). Persistence and Fate of Acidic Hydrocarbons in AquaticEnvironments: Naphthenic Acids and Resin Acids, Ph.D. Thesis, Univ. of Saskatchewan, Saskatoon, Canada.

McMartin, D.W., J.V. Headley, D.A. Friesen, K.M. Peru and J.A. Gillies. 2004. Photolysis of naphthenic acids in natural surface water. *Journal of Environmental Science and Health* A 39 (6): 1361–1383.

Mishra, S.,(2009). Microwave Assisted Photocatalytic Treatment of Naphthenic Acids in Water, Ph.D. Thesis, Univ. of Saskatchewan, Saskatoon, Canada.

National Energy Board of Canada (NEB). (2006). Estimated Production of Canadian Crude Oil andEquivalent. [Online]. Available fromhttp://www.neb.gc.ca/clfnsi/rnrgynfmtn/sttstc/crdlndptrlmprdct/stmtdprdctn-eng.html. Retrieved 2009-01-27[cited April 2011]

National Energy Board of Canada (NEB). (2011). Oil Sands. [Online]. Available from http://www.ercb.ca/portal/server.pt?open=512&objID=249&PageID=0&cached=true&mode=2 [cited April 2011]

National Energy Board of Canada (NEB). (2009). Total Crude Oil Exports (m3 and bbl) - Annual. [Online]. Available from http://www.neb.gc.ca/clfnsi/rnrgynfmtn/sttstc/crdlndptrlmprdct/ttlcrdlxprt-eng.html [cited April 2011]

Peng, J.; Headley, J.V.; (2002) Barbour, S.L. Adsorption of single-ring model naphthenic acids on soils. J. Can. Geotech. 2002, 39, 1–8.

Paslawski, J.C., M.Nemati, G.A.Hill, J.V. Headley (2009). Biodegradation kinetics of trans-4-methyl-1-cyclohexane carboxylic acid in continuously stirred tank and immobilized cell bioreactors. *Journal of Chemical Technology and Biotechnology*, *84*(7), pages 992–1000.

Paslawski, J.C., M. Nemati, G.A. Hill, J.V. Headley (2009).Model for biodegradation of naphthenic acid in an immobilized cell reactor. *Journal of Chemical Technology and Biotechnology*, *87*(3), pages 507–513.

Quagraine, E.K., H.G.Peterson, J.V.Headley(2005). In situ bioremediation of naphthenic acids contaminated tailing pond Waters in the Athabasca Oil Sands Region----Demonstrated Field Studies and Plausible Options: A Review. *Journal of Environmental Science and Health*, 40, 685-722. 82
Quail, B. E., Hill, G. A., (1991) A Packed-Column Bioreactor for Phenol Degradation: Model and Experimental Verification, J. Chem. Tech. Biotechnol. 52, 545-557.

Rogers, V. V., K. Liber and M.D. MacKinnon. 2002a. Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. Chemosphere 48: 519-527.

Rogers, V.V., M.Wickstrom, K.Liber, M.D. MacKinnon (2002b). Acute and subchronic mammalian toxicity of naphthenic acids from oil sands tailings. Toxicol. Sci. 66, 347–355.

Schindler, D.W. and Donahue, W.F., (2006), An impending water crisis in Canada's Western Prairie Provinces. Proceedings of the National Academy of Sciences of the United States of America, 103(19): 7210–7216.

Schmidt, E., M. Hellwig, and H.-J. Knackmuss. 1983. Degradation of chlorophenols by a defined mixed microbial community. Appl. Environ. Microbiol. 46:1038-1044.

Schramm, L.L.; E.N.Stasiuk,M. MacKinnon (2000). Surfactants in Athabasca oil sands slurry conditioning, flotation recovery, and tailings processes. In *Surfactants: Fundamental and Applications in the Petroleum Industry*; Schramm, L.L., Ed. Cambridge University Press: Cambridge, UK. 365–430.

Scott, A. C., M. D. MacKinnon and P. M. Fedorak. (2005). Naphthenic acids in Athabasca oil sands tailings waters are less biodegradable than commercial naphthenic acids. *Environ. Sci. Technol* 39: 8388-8394.

Scott, A.C., W.Zubot, M.D. Mackinnon, D.W.Smith, P.M. Fedorak (2008). Ozonation of oil sands process water removes naphthenic acids and toxicity . *Chemosphere*, 71(1), 156-160. Shell Canada. 2005. The Athabasca Oil Sands Project. 2004 Sustainability Report. [online]. Available from http://www.shell.com/static//caen/downloads/about_shell/what_we_do/aosp_sd_ report.pdf [cited 1 February 2006].

Shuler, M.L., Kargi, F., (2002). *Bioprocess engineering basic concepts*. Upper Saddle River, NJ, USA: Prentice Hall PTR.

Singer, P.C., and Reckhow, D.A.(1999). Chemical Oxidation. In Water Quality ad Treatment. A Handbook of Community Water Suppliers. American Water Association , McGraw-Hill, Inc., New York. No.12, p.12.1-12.51

Speight, J. G. (1999). Chemistry and Technology of Petroleum, Vol. 4, pp. 145-146. Marcel Dekker Inc, New York.

Stephenson, T., J. N. Lester, and R. Perry. 1984. Acclimatisation to nitrilotriacetic acid in the activated sludge process. Chemosphere 13:1033-1040.
Suncor Energy. (2005). Suncor Energy 2005 Report on Sustainability.[online]. Available from http://www.suncor.com/data/1/rec_docs/616_Suncor%20SD%20Report _2005%20.pdf [cited February 2011].

Syncrude Canada. (2010). 2010 Annual Tailings Plan Submission Syncrude Aurora [online]. Availablefromhttp://www.ercb.ca/docs/products/TailingsPlans/Syncrude_2010_AuroraNorth_Submission.pdf [cited 16 April 2011]

Syncrude Canada. (2004). Syncrude Canada Limited 2004 Sustainability Report. [online]. Available from http://sustainability.syncrude.ca/sustainability2004/download/ SyncrudeSD2004.pdf [cited February 2011]

Tanapat, S., (2001)" Comparison of the kinetics of biodegradation of geometric isomers of naphthenic acids (NAs) in Athabasca river water" M.Sc. Thesis, Univ. of Saskatchewan, Saskatoon, Canada .

U.S. Energy Information Adminstration (USEIA) (2011). Crude Oil and Total Petroleum Imports Top 15 Countries. [online] available from http://www.eia.gov/pub/oil_gas/petroleum/data_publications/company_level_imports/current/import.html [cited from April 2011]

Vidali M, (2001). Bioremediation. An overview. Pure Applied Chemistry. 73:1163-1172.

APPENDIX

A. Biomass Calibration

The concentration of biomass was determined by direct measurement of theoptical density (OD) of the samples taken from the flasks at a wavelength of 620 nm (Shuler and Kargi, 1992). An Ultraviolet (UV) spectrophotometer (Mini Shimadzu, Model 1240) was used for the determination of the optical density. The optical density was then related to dry-weight using a calibration curve presented in Figure A.1.

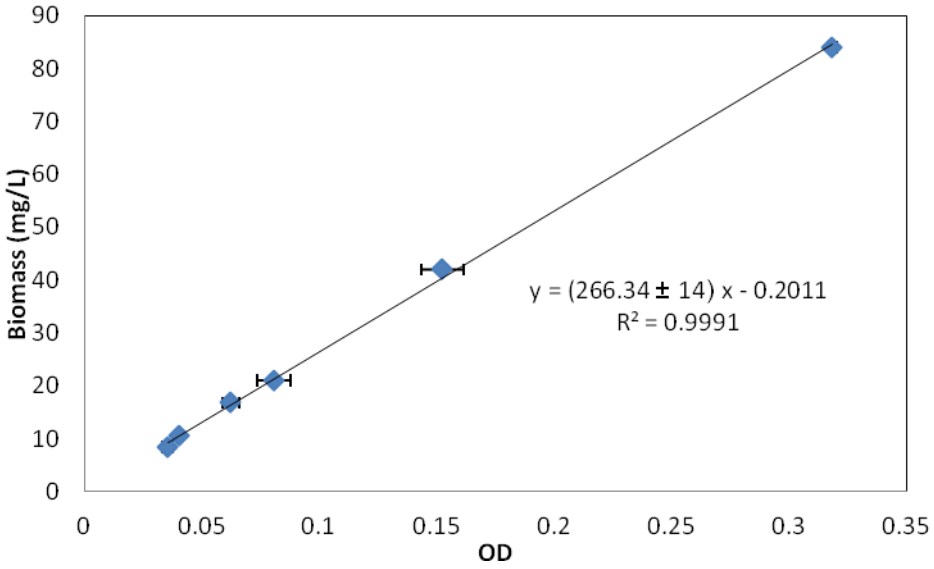


Figure A.1 Biomass calibration. Error bars represent standard deviation in optical density readings

B. Calibration Curves for Octanoic Acid

Utilization of GC-FID for direct analysis of model NAs in water and biological media requires a liner calibration curve to convert GC reading (uv.min) into actual concentration (mg/L). During the research standard solutions were prepared for octanoic acid. The generated calibration curves were updated regularly to ensure the accuracy of experimental results. The representative calibration curves for the model NA are presented through Figure B.2,

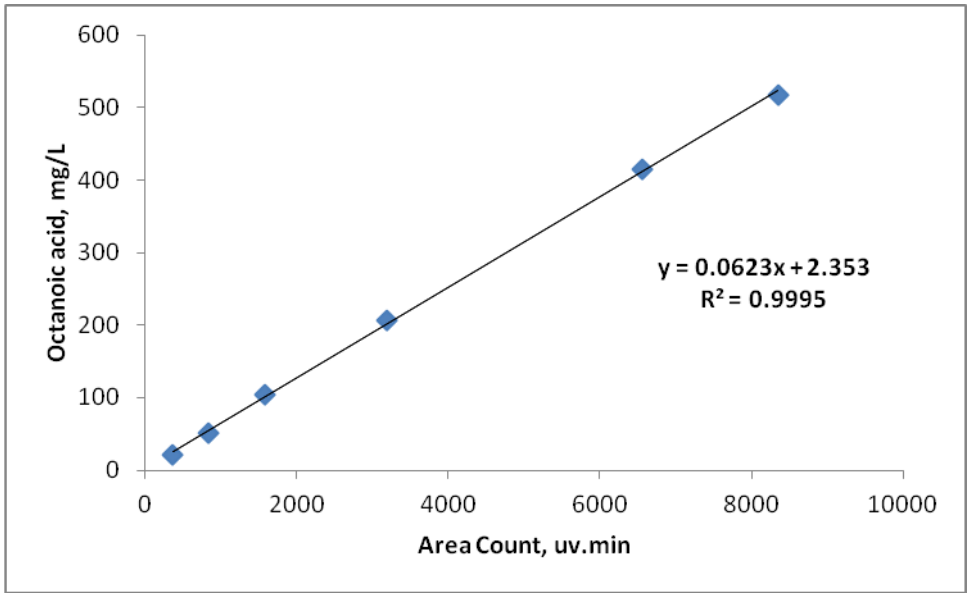


Figure B.1 The representative calibration curves for octanoic acid concentration measurement. Error bars represent standard deviation in GC readings and may not visible as the associated error is small.

C. Calculating Specific growth Rate

Specific growth rate was calculated according to the following equation.
$\ln (X_o/X_t) = \mu t$

Where,
X: biomass concentration in time t;
Xo: biomass concentration in time 0;
μ: specific growth rate;
t: time.

To calculate the specific growth rate, the biomass concentration data in the exponential growth phase were chosen and the value of ln (X/Xo) was calculated first. Then, ln (X/Xo) vs t was plotted and a straight trend line was passed. Finally, specific growth rate was calculated as the slope of the trend line.